A COMPARATIVE STUDY OF THE METABOLIC RESPONSE OF SMALL MAMMALS TO SELECTED ENVIRONMENTAL FACTORS: HYPOXIA, COLD AND VISUAL DISTURBANCE

A

DISSERTATION

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By
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HYPOXIA, COLD AND VISUAL DISTURBANCE

RECOMMENDED:

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ABSTRACT

The metabolic response to hypoxia was quantitatively defined in Microtus oeconomus under a series of metabolic loads (M_o). The critical ambient P_{O_2} for reducing O_2 uptake (P_c) was a linear function of M_o, thus $P_c = 73 + 13.2\ M_o$ (P_c in torr; M_o in met = multiples of the standard metabolism). Below P_c, the fractional reduction of M_o was 0.72%/torr, independent of the magnitude of M_o. The average P_c was lower in 7 highland species than in 15 lowland species and races of small mammals (110 vs 122 torr). Similarly the fractional reduction of M_o was also lower in the highland group (0.49 vs 0.75%/torr). Exposure to 80% He-20% O_2 increased M_o by 40 to 160% according to the extent and quality of the animal's insulation. In 10 species and races of homeotherms (including a passerine), lowering of ambient temperature elicited maximum metabolic rates in He-O_2 at 13 to 70°C warmer than in air. Deer mice showed a "freezing response" when alarmed by overhead shadow movements. The duration of this response varied between 8 minutes to longer than one hour. During this state as much as 60% decrease in ventilation and bradycardia of less than one third the normal heart rate caused a decrease in O_2 consumption to less than one half the normal values.

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INTRODUCTION

One hundred years ago Pflüger (1873) expressed the view that the animal cell itself determines its own respiratory exchange and that the oxidations were independent of the amount of oxygen supplied to it. This view was based on speculative teleological considerations and on the assumption that the oxygen supplied to the cells was normally in excess of their requirements. At the beginning of the century Thunberg (1905) applied the principles of chemical dynamics to the problem of oxidations taking place within the living body and concluded that variations in the oxygen supply should cause variations in the intensity of oxidations. According to his view, which was fundamentally opposed to that of Pflüger, the oxidative process should decrease with decreased oxygen pressures and increase with increased pressures to gradually approach a maximum rate. Although close to modern views, Thunberg looked upon the catabolism of nutritive materials as a simple process of oxidation. Actually, at each of the breakdown stages the reaction proceeds at a certain rate depending not only on the number of molecules taking part in it but also on the specific velocity of the reaction in question. Also, the reaction velocity of the whole process will be determined by the slowest of the partial reactions. Oxygen will become a "limiting factor" only when the reaction(s) in which the molecular oxygen is involved becomes the slowest of the chain. Until then, changes in oxygen concentration will have very little influence upon the velocity of the whole process at the cellular

level. Technological difficulties are encountered in attempting to determine the concentration at which oxygen becomes the limiting factor in the oxidative process, due to the fact that the determinations of P_{O_2} should be conducted in the cells in which the oxidations occur. Nevertheless, measurements of oxygen tension and consumption have been conducted in whole organs as in brain of rats (Eklof et al., 1973) or in leg muscles of cats and guinea pigs (Vetterlein and Schmidt, 1972; Whalen and Nair, 1967). These measurements however are influenced by changes in blood flow, or in systemic blood pressure and vascular resistance (Doll et al., 1968; Ote, 1969). Moreover, as the particular P_{O_2} value will also depend upon such factors as diffusion distance between the capillary and the cells and the metabolic rate of the particular tissue (Rahn, 1966), the unknown distribution of tissue areas metabolizing at different rates suggests some uncertainty in the estimation of P_{O_2} at the cellular level with whole organ perfusion techniques. Extrapolation of conclusions based on in vitro observations to the hypoxic response in vivo may be misleading. Thus although the redox state and O_2 consumption of brain mitochondria in man remain normal when $P\hat{a}_2$ is as low as 4 torr, unconsciousness supervenes when venous P_{O_2} is lower than 18 torr (Cohen, 1972). The whole organism presents on the other hand an alternative at a higher level for the study of the relations between environmental P_{O_2} and oxidation rates. The early experiments of hypoxia on whole organisms such as man and laboratory mammals conducted by Speck (1892), Loewy (1895), Durig (1903),

Zuntz (1906) and Hasselbalch (1914) showed that the total pressure could be diminished to 400 torr (P_{O_2} of about 84 torr) without any perceptible influence on the rate of O_2 consumption. By a further diminution of P_{O_2} a slight increase in O_2 consumption was observed, which however, when corrected for the increased work of the respiratory muscles, resulted in a final slight decrease of metabolic rate. Krogh (1916) summarized these early observations on the effects of P_{O_2} on metabolism of mammals by indicating that the limit at which the oxygen absorption begins to fall lies somewhere between the P_{O_2} of 90 and 75 torr in the inspired air thus virtually introducing the concept of a critical pressure of oxygen (P_c), a term utilized a few years later by Tang (1933), who described it in relation to hypoxic environments as the point where an increase in O_2 tension produced no effect on the rate of O_2 consumption. In recent years Prosser and Brown (1961) and Cohen (1972) defined P_c as the pressure of oxygen below which O_2 consumption decreases. Determinations of P_c require rather simple technology and one would expect a larger number of data in different species to be on hand for comparative studies. Nevertheless the information on this area is scarce and moreover conflicting; a P_c of 32 torr has been given for Rattus norvegicus and Mus musculus (Hall, 1966) while from the data of Adolph and Hoy (1960) a P_c of 92 torr can be calculated for rats, and Giaja (1946) reported a P_c of 85 torr for white mice. This discrepancy can hardly be thought due to differences in basic methodologies or

to differences in the individual animals utilized, but mainly to some other factor affecting the P_c values. One possible factor which could explain these results is the metabolic level (M_o), at which hypoxia is given, as suggested by Prosser and Brown (1961). The first chapter of this thesis is devoted in great extent to the study of the influence of the metabolic load on P_c as well as on the metabolic reduction during hypoxia. For this purpose a single species of rodent, Microtus oeconomus, was utilized. Quantitative relations obtained in this species subjected to different degrees of hypoxia, have then been applied to a larger number of species native from low and from high elevations and exposed to hypoxia in a similar way to the tundra voles. In these experiments a high metabolic load was imposed by lowering the ambient temperature so as to elicit about 4 times the standard metabolic rate. In addition respiratory frequency measurements at thermoneutrality in normoxic as well as in hypoxic conditions gave an index of hypoxic sensitivity. The second chapter in this thesis is devoted to this comparative study.

Both hypoxia caused by lowering the environmental P_{O_2} or by exhaustive metabolic demands in a normoxic atmosphere are characterized by a common factor which is a tissue O_2 supply lower than required. Hypoxic exposure reduces the oxidative capacity of muscle mitochondria in the same manner as that found with heavy exercise, thus both hypoxia and high O_2 demands are thought to be mediated through a common process (Dohm et al., 1973). Moreover, adaptation to hypoxia can be induced by lowering the inspired air P_{O_2} as well as by the performance of strenuous muscular work in normal air (Barbashova, 1967). With the exception of

white mice and rats and two or three other species such as Peromyscus leucopus and Microtus arvalis, little is known on the maximum metabolic capability (M_{max}) of small mammals. Technological difficulties as sub-freezing temperatures, treadmills and training for running, conditions that the conventional methods require, may explain this lack of information. The third chapter of this thesis refers to a new methodology developed for this purpose, which obviates most of the undesirable qualities of the commonly used methods. Based on the high thermal conductivity of an atmosphere of 80% helium and 20% oxygen (about four times higher than air) and in conjunction with moderate cold exposures, maximum response to heat loss was elicited at up to 70°C warmer temperatures than those required in normal air for a similar metabolic effort. This and other important advantages seem to indicate this technique as the future method of choice for determining maximum metabolism or lethal thermal minimum values in small homeotherms with no apparent damage to the animals. M_{max} values obtained with this technique in a series of small homeotherms and in normoxic atmospheres are also reported in this section, but M_{max} values obtained during hypoxia are discussed in Chapter 2 which deals more with the comparative aspects of exposures to low P_{O_2}.

Oxygen deficiency can occur not only when P_{O_2} is lowered in the inspired air or during heavy exercise in normal air, but also when resting in a normoxic environment. Ventilatory insufficiency in emphysema (VanLiere and Stickney, 1963) or hypoventilation during metabolic alkalosis can result in arterial P_{O_2} values as low as 52 torr

(Shear and Brandman, 1973). Hypoventilation and bradycardia leading to a marked reduction in oxygen consumption was observed during our experiments with deer mice when these rodents were stimulated by a moving shadow. The reflex development of hypoxia in a normoxic atmosphere was considered of sufficient biological interest as to justify a basic characterization of this behavioral response. Chapter 4 is devoted to this problem and includes respiratory, circulatory, as well as metabolic and body temperature measurements during this condition.

The different chapters of this thesis have been written following the instructions for preparation of manuscripts of the American Journal of Physiology or Journal of Applied Physiology (Chapters 1 and 3), Comparative Biochemistry and Physiology (Chapter 2), and Physiological Zoölogy (Chapter 4). The broad scope of the chosen journals embraces the basic styles of most journals in physiology-zoology.

Chapters 1, 3, and 4 have presently been accepted for publication.

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CHAPTER 1

PHYSIOLOGICAL RESPONSES TO HYPOXIA IN THE TUNDRA VOLE

INTRODUCTION

The metabolic uptake of oxygen in mammals is limited by sufficiently hypoxic environments, and an increase in metabolic rate by exercise or by cold exposure accentuates this limitation, i.e., increases the critical partial pressure of oxygen below which O_2 consumption can no longer be maintained (72).

Because of the inherent problems of human experimentation, determinations of critical pressures in man are difficult to obtain. Alveolar pO_2 during rest has been reduced to 60 Torr before steady-state ventilation showed a significant increase (46), and collapse during hypoxia has been reported to occur at arterial partial pressures below 25 Torr (60), equivalent to >29,000 feet (42). Although these elevations are beyond the exposure levels of mountaineers, their metabolic effort may be several times that at rest, so that critical limits are reached at much lower elevations.

In contrast to man, different species of mammals provide a range of models for relating physiological architecture and performance at high altitude. In this respect critical values, at 5°C were found to be lower in rodents native to high altitude than in lowland species (56) and excepting some qualitative observations in laboratory and white-footed mice (17,72) little is known of the degree by which O_2 consumption is impaired when various levels of hypoxia are imposed at different metabolic levels. A variety of methods have been applied by different authors to assess hypoxic sensitivity or its reciprocal--resistance or

tolerance--from rather modest to very severe hypoxia. Some criteria are irreversible like gasping time (44,53), or survival time (10,27,62,73). Less drastic tests include righting times (7), increase in ventilation (49,74), erythropoietic response (52), retention of position in an inclined plane (68), minimal utilizable O_2 (39), ability to rewarm after hypoxic hypothermia (41), etc.

In this study our primary measure of hypoxic involvement was the response of metabolism to pO_2 as measured at different metabolic loads but associated changes in respiration, heart rate and body temperature were also observed.

METHODS

The tundra vole, a small northern microtine rodent, was taken as a representative lowland species. This use of a naturally occurring form avoids the selective influence of man on the common laboratory animals. Adult specimens of both sexes of Microtus oeconomus tananaensis were collected near Fairbanks, Alaska, and kept at 5°C, 18°C or 22°C for periods of 3 to 5 weeks prior to study. Animals which were born and raised in captivity were also used.

Oxygen consumption was measured in individually caged specimens with an automatic manometric respirometer (55), which maintained a fixed pO_2 within $\pm$ 3 Torr. The metabolic chambers were kept under water in a large thermoregulated bath. The water vapor pressure was reduced with $CaCl_2$ to avoid reported effects of humidity on hypoxic resistance (62).

Hypoxic atmospheres were obtained by accurate dilutions of air or oxygen in nitrogen or helium by Godart gas mixing pumps. Changing the pO_2 usually took less than 12 minutes and the desired atmosphere was held for periods of 1 to 9 hours, depending on the nature of the experiments. During determination of maximum metabolism (M_{MAX}) in hypoxia, (67) peak metabolic rates were reached in less than one hour. The lowest level of inspired pO_2 tested was 38 Torr equivalent to an altitude of about 35,000 feet but in most tests pO_2 was only lowered to 54 Torr.

Results obtained by exposures to gas mixtures at 750 mm Hg, are similar to those obtained using altitude chambers or at real altitude. Thus, total nitrogen excretion, urea excretion, blood lactic acid concentration as well as concentrations of urea in serum, liver glycogen and blood glucose have been reported to be affected in an identical manner when rats were exposed to either reduced total pressure or to equivalent oxygen-nitrogen mixtures (69). On the other hand specific effects of low pressure (35,000 feet) without hypoxia, have been reported to result in a reduction of forced vital capacity in man (77). Similar observations in rats have failed to demonstrate any effect of low pressure per se on rate of growth, O_2 consumption, food and water intake, hemoglobin concentration or behavior (25).

Different levels of energy demand were obtained by changing the ambient temperature (T_A), and maximum and near maximum metabolism was elicited by a combination of cold and helium-oxygen gas mixtures (67). The influence of hypoxia was evidenced by a reduction of O_2 consumption

below the level required for thermoregulation. The reduction in body temperature (T_B), was measured at the end of the experiments or was monitored continuously in some animals by radiotelemetry from implanted transmitters (59).

Pulse rates were obtained through electrodes connected to the floor of an animal cage which was made of parallel copper wires that were brushed with cardio-paste before each experiment. A pair of multiple switches were used to relocate the electrical signal when the animal stepped to a different position in the cage. Reported difficulties with small mammals and hard-wire recording leads (54) were avoided in this way. Respiratory frequency was measured with a pressure transducer connected to the metabolic chamber and a 2-channel DC-AC Gilson EEG recorder which also received the ECG imput.

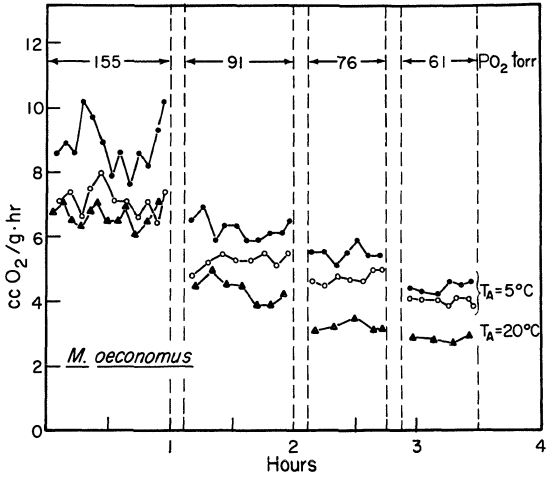
In tests of 1 to 2 hours duration, no food was given to the animals, but in longer experiments sunflower seeds and pieces of apples and carrots were provided. To facilitate comparisons between animals of different body size, O_2 consumption was also expressed as a multiple of the standard (mean basal) metabolism for mammals:

1 Met = 3.8 $W^{-.73}$ (cc O_2/hr, g).

RESULTS

The metabolic effect of exposing tundra voles to progressively lower pO_2 is shown in Figure 1 as a function of time. It may be noted that O_2 consumption is stable during each 40-60 minute exposure but

Fig. 1 Representative metabolic experiments on three tundra voles with 40 to 60 minute exposures to progressively lower pO_2.



shows a direct relation to pO_2. The linear nature of this relation is shown in Figure 2 which plots mean values for O_2 consumption (mets) against pO_2 representing reductions to 66-26% of the value in normal air.

Since the animals were in negative heat balance throughout this hypoxia, the T_B fell continuously and this substantial ensuing hypothermia might be influencing the metabolism as well as the hypoxia. Accordingly, a second set of tests were performed 3 weeks later on the same animals and under the same hypoxic conditions but with a one hour recovery at normal pO_2 after each hypoxic exposure. Although the normoxic and hypoxic rates in the second series were slightly higher than in the previous test, the degrees of metabolic depression were identical (Fig. 2). This independence of metabolism and T_B is a striking phenomenon intrinsically and was of great importance in our experimental design since the hypoxic responses were not compromised by varying T_B.

To examine these effects in more detail, animals instrumented with T_B transmitters were exposed for a number of hours at 5°C ambient temperature while O_2 consumption and T_B were continuously monitored. The results of typical experiments are shown in Table 1. Hypoxic exposure at 83 Torr (~17,000 ft.) caused an average 30% decrease in O_2 consumption that kept constant within $\pm$6% in spite of a progressive 5-hour fall in T_B to about 30°C. However, below 30-31°C O_2 consumption did decline following further reduction in T_B.

During exposure to lower concentrations of oxygen, at 54 Torr (~27,000 ft.) the average reduction was 43% and was maintained within

Fig. 2 Decrease in O_2 consumption during successive exposure of 30 to 45 minutes at reduced pO_2 (closed circles), and with one hour recovery periods at room pO_2 between hypoxic steps (open circles). Vertical lines indicate range of values.

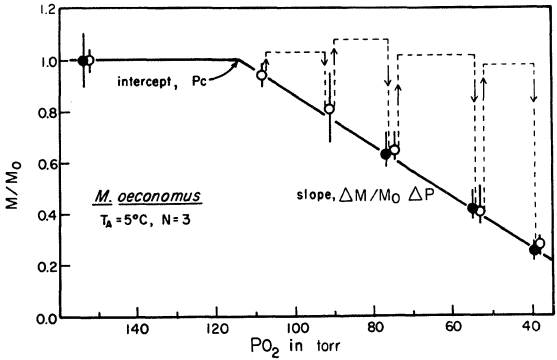


TABLE I: Metabolism and body temperature in hypoxic tundra voles at 5°C

pO_2 Torr	Time Hrs.	M $cc O_2/g\ hr$	M/M_0 (%)*	T_B
155	control	5.3-7.5	100	38.6 ± .2
83	0.1	4.0	63	38.0
83	1.0	4.7	74	36.3
83	2.0	4.5	70	35.6
83	3.0	4.5	70	34.7
83	4.0	4.5	70	33.2
83	5.0	4.0	63	31.2
83	5.3	4.7	74	30.4
155	control	6.2-8.0	100	38.3 ± .3
54	0.25	4.1	58	34.8
54	0.33	4.2	59	33.3
54	0.41	4.2	59	32.4
54	0.50	3.9	55	31.2
54	0.63	3.8	54	29.9

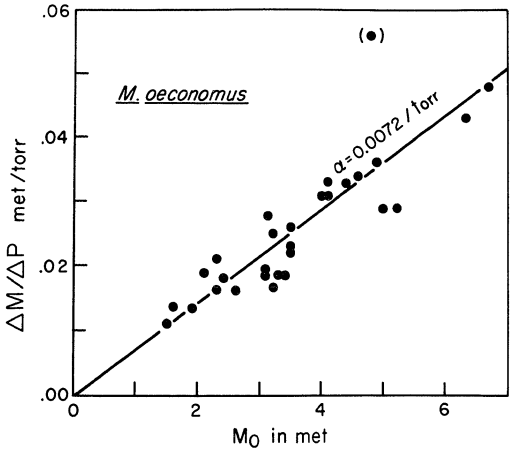
* Estimated as % of the average rate during 1 to 2 hrs of pre-hypoxic measurements (control).

±3% while the body temperature dropped to 30°C within 40 minutes. Thus, under more severe hypoxia the same independence of metabolism and T_B was observed but the rate of hypothermic induction as well as the reduction in metabolism were enhanced.

Observations of the same nature in other species of rodents (Phyllotis, Dicrostonyx, Peromyscus) have given similar results (Rosenmann and Morrison, to be published). Although the level of hypoxic metabolism was different in each species, reductions in T_B did not affect the rate of oxygen consumption during hypoxia. From these observations it is obvious that the constant rate of heat production during hypoxia does not represent an equilibrium in energy balance but rather a constant deficit which constitutes one measure of hypoxic impairment. Since values at different pO_2 presented a linear array (Fig. 2), the slope of the curve provides a convenient index of this effect. The other index of the hypoxic response is the critical pressure, P_c, the lowest tension of oxygen tolerated without metabolic reduction.

Since the hypoxic rate of O_2 consumption was obviously modified by the initial metabolic intensity, this influence was systematically explored in a series of experiments on 29 voles exposed to different hypoxic atmospheres and under graded metabolic loads ranging from about 85% of maximum down to near basal metabolism. The slopes of these curves of M vs. pO_2 were computed and are presented in Figure 3 as a function of initial metabolic level ($\Delta M/\Delta P$ vs. M_o). These in turn form a linear array and can thus be summarized by a single

Fig. 3 Relation between the metabolic depression (mets/Torr) and the initial metabolic intensity (M_o). Each point represents a different individual and the indicated curve shows the average ratio for the 28 voles, 0.0072 $\pm$ 0.0011/Torr.



constant, α, representing the fractional reduction in M_o per Torr:

$$\alpha = d(M/M_o)/dP = 0.0072 \text{ Torr}^{-1} \quad (1)$$

$$\text{or } \alpha = 1-(M/M_o) \; / \; P_c - P \quad (2)$$

In like manner when P_c is plotted against pO_2 (Fig. 4) these data may also be represented by a linear curve up to a metabolic level of 4.5 mets:

$$P_c = 73 + 13.2 \; M_o. \quad (3)$$

By combining equations (2) and (3) we can describe the metabolism limited by hypoxia as a function of the initial metabolism (N metabolic stimulus) and the pO_2:

$$M \; / M_o = 1 - 0.0072 \; (73 + 13.2 \; M_o - P) \quad (4)$$

$$\text{or, } M \; / M_o = 0.47 - 0.095 \; M_o + 0.0072 \; P \quad (4a)$$

Thus, M/M_o as a function of pO_2 presents a series of parallel contours for values of M_o (Fig. 5). When the metabolic level is plotted directly against pO_2, the values may be seen to converge near p = 30 Torr and 1 Met (Fig. 6). All pO_2 values below P_c result in some hypoxic interference with normal function. However, limited interference may not compromise the individual seriously. Thus, for a tundra vole at 5°C with M = 4 Mets, T_B might stabilize at 32° for a pO_2 of 99 Torr.

The implications of the influence of the initial metabolic level, or more properly the metabolic stimulus, are of interest. The animal goes into thermal deficit even though it has adequate respiratory capacity to maintain equilibrium, thus showing a deficiency in control

Fig. 4 Influence of the initial metabolic intensity (M_o) on the critical pressure (P_c). The regression 73 + 13.2 M_o (standard error of estimate, 3.0 Torr) was determined in a total of 36 voles acclimated at 18°C (closed circles), 22°C (square), and at 5°C (open circle).

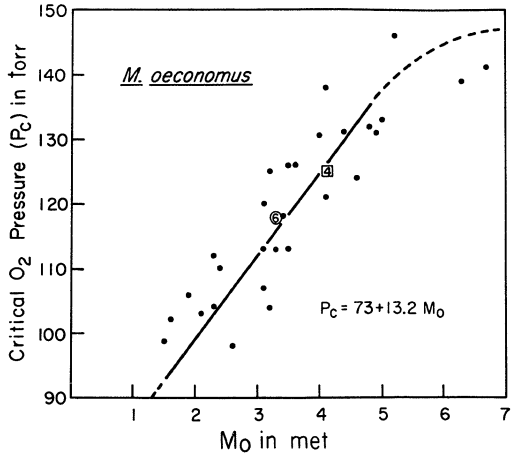


Fig. 5 Fractional metabolic reduction (M/M_o) in relation to pO_2 and metabolic load (M_o). Arrows indicate P_c for each load.

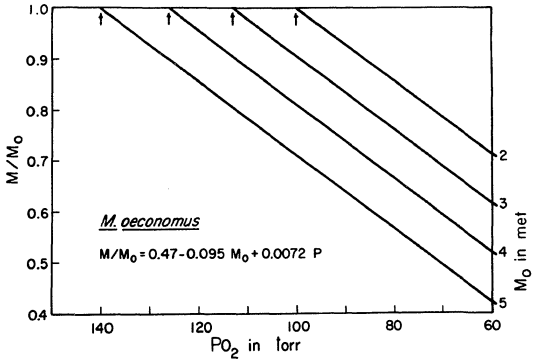
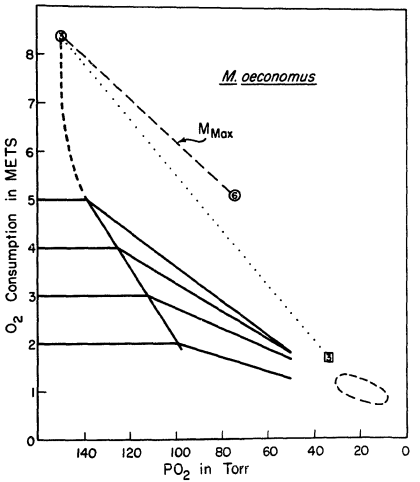


Fig. 6 Metabolic responses to pO_2 at different loads. Maximum O_2 consumption at 150 Torr and at 74 Torr (T_A = 1°C in He/O_2) indicated by circles. Minimum (lethal) P_c values at T_A = 28°C indicated by square. Dashed area represents zone of minimum P_c for other species of rodents (39).



function. This is further emphasized by the two points in Figure 6 representing maximum metabolism. At pO_2 of 74 Torr M_{MAX} was twice that elicited by T_A of 5°C.

Our data of the effects of hypoxia show increases in respiratory frequency in M. oeconomus as shown in Figure 7. A 100% increase in respiratory frequency was observed during the first 4 hours of hypoxic exposure and this elevated rate was maintained despite a progressive fall in T_B to 32°C. This maintenance of rapid respiration during a 7°C temperature drop contrasts with the reported dependence of breathing rates on T_B (18). On the other hand it can partly explain the maintenance of O_2 consumption during hypothermia by an overriding of temperature effects by the hypoxia ventilatory drive. Although at T_B of about 30°C the respiratory frequency still showed an increase of 75 to 80% with deeper hypothermia, O_2 consumption and respiratory frequency declined together with T_B.

In contrast to respiration, exposure to hypoxia caused a decrease in heart rate. Figure 8 compares the progressive bradycardia with the constant metabolic depression at two different levels of pO_2. Thirty minutes at a pO_2 of 54 Torr resulted in a 30% drop, while at 93 Torr the reduction in equal time was only 17% of the pre-hypoxic rates. Considering a resting metabolic rate of about 6.8 $ccO_2(g\ hr)^{-1}$ at 156 Torr, the metabolic depression was 35% at the lowest O_2 exposure and 17% at 93 Torr.

After three weeks of acclimation to 5°C and to 22°C, the respective groups of M. oeconomus failed to demonstrate any significant effect on

Fig. 7 Respiratory frequency (open circles), body temperature (closed circles) and oxygen consumption (heavy line) in a male vole during hypoxia at T_A = 6°C.

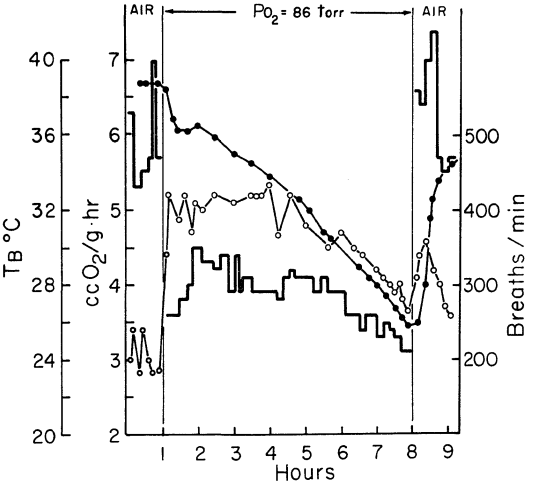
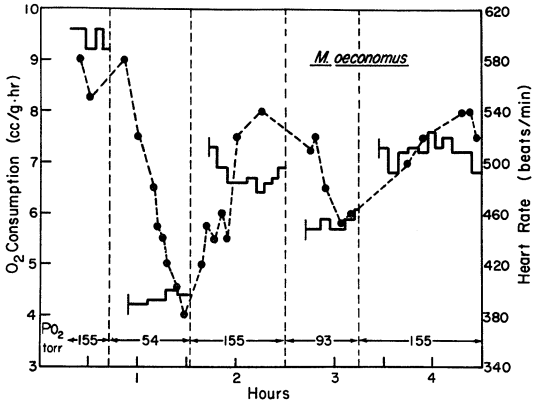


Fig. 8 Heart rate (closed circles) and O_2 consumption (heavy line) during hypoxic exposures to 54 and 93 Torr at T_A = 5°C.



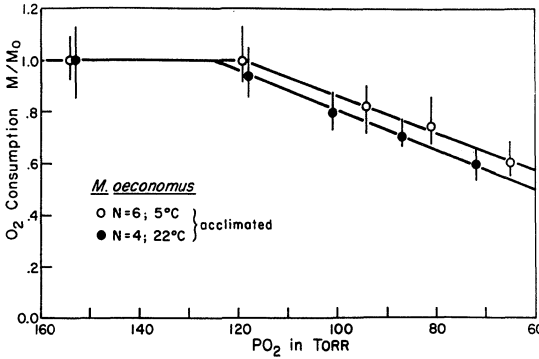
the metabolic response to hypoxia (Fig. 9). Probably due to their larger size the cold acclimated group showed a lower metabolic rate than the 22°C group when measured at 5°C. Nevertheless, their P_c values and hypoxic slopes correspond well with data in Figure 4 when adjusted for metabolic level.

DISCUSSION

Oxygen consumption under hypoxia.

Physiological regulators are distinguished from conformers by their efforts to maintain rates or levels despite moderate ambient changes in the operational variable. Thus, the tundra vole, like most mammals (1) can sustain considerable reductions in pO_2 (to the P_c) with no reduction in metabolism and the value of this P_c provides a measure of effective regulation or resistance to hypoxia. However this measure is influenced by the initial metabolic load or stimulus so that the higher the load, the greater the sensitivity. This influence of T_A or metabolic level may have led to apparent contradictions as reported for the minimal pO_2 in chinchillas vs guinea pigs (27, 39) or with respect to the ability of rats vs. mice to survive hypoxia when lowering T_A below 30°C (10,62). Below the P_c the metabolism can change sharply or more gradually with further reductions in pO_2 to provide a second measure of hypoxic sensitivity but fortunately this measure was independent of metabolic load when expressed in relative terms (M/M_o vs pO_2).

Fig. 9 Fractional metabolic reduction in 5°C and 22°C acclimated voles exposed to hypoxia at T_A = 5°C. Vertical lines indicate range of values. Initial metabolic intensities and P_c values for both groups are shown in Fig. 4.



Both relations (equations 2 and 3) should be useful in normalizing data obtained under somewhat different conditions to compare the effect of other variables in the tundra vole (e.g. cold acclimation). Further, we might expect the form of these equations to apply to other species of small mammals although we would not expect the same values for the constants. Earlier studies have shown that oxygen consumption was reduced by a sufficient degree of hypoxia, resulting in death or irreversible changes at high T_A or in hypothermia at low T_A. Thus Chevillard (17) summarized his earlier observations in 1935-7 on white mice by relating pO_2 and M_o, indicating that the lower the pO_2 and the higher the initial metabolism, the greater the metabolic deficit and the corresponding degree of hypothermia. Similarly, Segrem and Hart (72) showed in the white-footed mouse that as T_A was lowered from 20 to -20°C, P_c increased progressively from about 60 to 150 Torr. These observations correspond qualitatively with our findings on the tundra vole, but unfortunately these data on both the white mouse and the white-footed mouse are not extensive enough to define numerical indices for the two constants, α and P_c.

Hypoxic hypothermia and metabolic rate. One of the most significant findings in the response of M. oeconomus to hypoxia was the independence of metabolic rate and T_B within a range of 38°C to approximately 30°C. Although this phenomenon has been previously observed in other species, it has not been sufficiently emphasized. Oxygen consumption in hypoxic dogs showed little change during a reduction of T_B from 37.5° to 35°C

(51). Hypoxic rats have been reported to maintain the rate of O_2 consumption in spite of a 3°C drop in T_B (35). Hypoxic ground squirrels have been observed to keep a constant O_2 consumption (34 $\pm$ 4cc/kg·min) while T_B dropped 5°C in one hour (14). Exposure of dogs to 6% O_2 caused a drop of T_B from 39° to 34°C in about 4 hours, during which time O_2 consumption was held at 150 $\pm$ 20 cc/min (60). Independence of these two parameters has been also reported in hypoxic young rabbits (78), and also suggested in normoxic men during exercise, within a range of rectal temperatures of 38° to 35.4°C (79).

The following factors may contribute to the maintenance of metabolism in hypoxia despite hypothermia:

(a) respiration is maintained constant until T_B approaches 30° to 32°C;

(b) activity is largely suppressed under hypoxia leading to a more stable rate of O_2 consumption. (A few struggling voles became hypothermic at a faster rate and did not maintain O_2 consumption for as long as inactive ones.)

(c) a shift of the O_2 dissociation curve of Hb to the left will be expected in proportion to the hypothermia which in turn relates to the degree of hypoxic exposure.

At 5°C the rate of cooling in our voles was 2°C/hr at pO_2 of 83 Torr as compared with 13°C/hr at 54 Torr. Resistance of rodents to hypoxia has been ascribed to the decrease in T_B (4), and to the resulting increased affinity of Hb for oxygen (24). A T_B drop of

3°C in hypoxic dogs caused an increase in saturation from 85% to 91% (60), and in hypothermic woodchucks venous pO_2 has been shown to increase 35% for a 5°C drop in T_B (32). The "protective" role of hypothermia in resisting or surviving hypoxia has been shown in rats (33,76), cats (33,37), rabbits (12), mice (38), and in dogs (33). This has been attributed to diminished cerebral O_2 demands and to the smaller changes in cerebral concentration of ATP, ADP, phosphocreatine and lactate produced under hypoxic hypothermia (19).

Heart rate during hypoxic exposure. Bradycardia in relation to hypoxia has been reported in mice (57), rabbits (6), rats (2,3,23), dogs (9,22,40), woodchucks (16), ground squirrels (13), and in man (61); but the opposite response, tachycardia, has also been reported in man and different species of homeotherms (1,21,26,48,50,58). In addition, an unchanged heart rate under hypoxia has been reported in man (47), and in rats (30).

One factor that may partly explain this discrepancy at least in species smaller than man, is the different degree of hypothermia during hypoxia which is related to the intensity and the duration of the hypoxic exposure. As Q_{10} values for heart rates of dogs, rabbits, and rats have been reported to be between 3.0 and 4.2 (8,11,20) just one degree change in T_B should result in about 14% change in heart rate, assuming an average Q_{10} of 3.5. A second factor may be different durations of hypoxia. Acute exposure of men to simulated 18,000 ft resulted in a 3 to 9 beats/min increase in heart rate in 25 to 35

minutes, but a slight reduction of 1 to 3 beats/min below the pre-exposure values was observed after 60 to 90 minutes (36). A third factor is the degree of hypoxia. An increase of the heart rate has been observed in hypoxic dogs down to 75% of arterial saturation, but below this value the response changed to bradycardia (21). Similarly in normothermic but hypoxic rats a 25% tachycardia was observed down to a pO_2 of 56 Torr, below this level bradycardia developed in proportion to the shortage of oxygen (1), and a 20% increase of heart rate was seen in rats exposed to 13,000 ft but a marked bradycardia was observed at higher simulated altitudes (2). A fourth factor influencing the heart rate response to hypoxia is the metabolic load (M_0). Rats and ground squirrels increased their heart rate 16 to 20% when exposed to hypoxia at low metabolic loads, but bradycardia of 10 to 35% developed at the same pO_2 and time under high metabolic requirements (15).

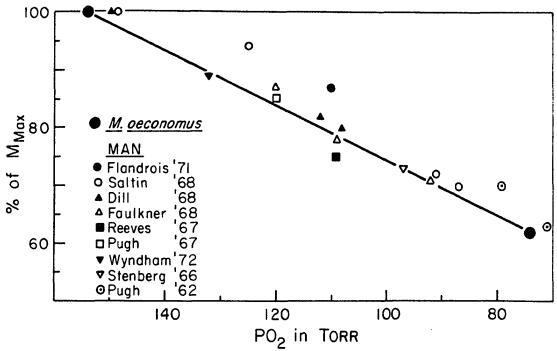
Our tundra voles showed no immediate reduction in heart rate on hypoxic exposure but a continuous decline throughout the period in proportion to the metabolic depression or caloric deficit. The Q_{10} estimated for these changes is comparable to those noted above for other species. Thus hypothermia may be viewed as the main cause for the observed bradycardia. Nevertheless these experiments conducted at 4-7 mets do not rule out the possibility of a different response at lower metabolic loads. A tachycardic response to hypoxia from sea level down to P_c and a bradycardic effect below P_c may also be a possibility.

Respiratory frequency during hypoxia. Exposure of different species to low pO_2 has been reported to cause an increase in respiratory frequency, tidal volume, or ventilation (3,16,22,70,74). Our data on the respiratory frequency of the tundra vole under hypoxic conditions showed similar effects, the high rate of respiration being unaffected by hypothermia of 7°C. However, below 30°C the respiratory frequency declined following the fall in T_B. Comparable results have been reported in hypoxic rats in which a 50% increase in respiration was observed up to 23,000 ft, but a marked reduction was recorded at higher elevations (3).

Cold acclimation and the metabolic response to hypoxia. Resistance to hypoxia has been reported to be affected by thermal acclimation. Decreased tolerance to hypoxia after cold acclimation has been observed in rats (5,10,31), and heat acclimation has increased the anoxic resistance of white mice (43). Cold acclimation has also been reported to increase the resistance of rats to hypoxia (34), and in the same species a positive cross acclimation between altitude and cold has been indicated (76). Roberts, et al. (66) compared the effects of cold acclimation on some dozen biochemical indices of metabolism with the differences between native deer mice from 3.8 km and from sea level. A good cross correlation between cold and altitude was seen in three or four of these factors not including oxygen consumption for liver and kidney homogenates nor basal metabolism of intact animals. We found no effects in a three week acclimation at 5°C (vs 22°C) but a longer acclimation or a greater difference in temperature might have elicited a response.

Maximum metabolism under hypoxia. Although some studies have been conducted in Peromyscus on the ability to perform work (maximum effort) or to sustain work (endurance) under hypoxia (72,45), similar information for other species of rodents is not available. Determinations of M_{MAX} at pO_2 of 154 Torr and at 74 Torr in the tundra voles showed a reduced metabolism but to lesser degree than seen with lower metabolic stimulus (0.48 vs 0.72% M_o/Torr). However, comparing metabolism at the limiting pO_2 (35 Torr @ 28°C) gave a mean reduction of 0.68% M_{MAX}/Torr close to the mean value for α (Fig 6). Although data of M_{MAX} at intermediate levels of pO_2 are obviously desirable, the relationship between pO_2 (or altitude) and M_{MAX}, looks so far in M. oeconomus strikingly similar to the values reported for man (Fig. 10) so that the utilization of this species as a model of human performance at high altitude may be appropriate.

Fig. 10 Reduction of maximum O_2 consumption at low pO_2 levels. Curve and large circles are for M. oeconomus. Values reported for man by different authors are shown for comparison.



SUMMARY

The metabolic response (M) to hypoxia was measured in the tundra vole, Microtus oeconomus, under a series of thermal metabolic loads (M_o) and hypoxic atmospheres with pO_2 (P) ranging from 150 to 54 Torr. At each M_o, metabolism was maintained down to a critical pressure (P_c) which was a linear function of M_o, thus, $P_c = 73 + 13.2\ M_o$ (P_c in Torr; M_o in mets). Below the P_c, M was a linear function of P falling in proportion to M_o so that $\Delta M/M_o \Delta P = 0.0072$ and $M/M_o = 0.47 - 0.095\ M_o + 0.0072\ P$. Hypothermia of up to 8°C during hypoxia did not modify O_2 consumption but heart rate was markedly depressed. Hyperventilation and a likely increased affinity of hemoglobin for O_2 may contribute to this independence of body temperature and metabolic rate. No difference in hypoxic sensitivity was observed between voles acclimated at 5°C and 22°C. Maximum O_2 consumption was reduced in hypoxia in about the same proportion as reported for man at high altitudes, 38% at 6 km.

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CHAPTER 2

METABOLIC RESPONSE OF HIGHLAND AND LOWLAND RODENTS TO SIMULATED HIGH ALTITUDES AND COLD

INTRODUCTION

Adaptation to high altitude involves one or more steps in the transport chain of oxygen from the surrounding atmosphere to its final utilization in the tissues (Dill, 1938; Morrison, 1964). Although the identification of specific adaptations as in erythrocyte mass, degree of vascularization, myoglobin concentration, etc. has been of evident value, it is the overall metabolic response to hypoxia which gives the integrated measure of the degree of adaptation to high altitudes. Near basal conditions, highland rodents are more resistant to hypoxia than lowland species (Morrison, 1964; Hall, 1966), but observations at higher oxygen demand which better approximates natural requirements are less available.

As "regulators" mammals can sustain some degree of hypoxia without modifying their oxygen consumption but at some "critical" P_{O_2} metabolism will be reduced. This critical pressure (P_c) is a measure of the hypoxic sensitivity of the species but has also been shown to be related to the level of oxygen demand in the individual (Segrem & Hart, 1967; Rosenmann & Morrison, 1974a). As the P_{O_2} is lowered below the P_c, metabolism (M) is progressively restricted until a lethal level is reached. In the tundra vole this incremental reduction is linear and independent of the level of oxygen demand (M_o) when expressed in relative terms:

$$(1/M_o)\ (DM/dP) = \alpha.$$

This hypoxic coefficient, α, constitutes a second measure of sensitivity to altitude.

In the present study we assess the hypoxic sensitivity of a variety of highland and lowland rodents at a fairly high level of oxygen demand according to these two indices to provide a measure of adaptation in their respective environments. We also compare the respiratory response to hypoxia in these species.

MATERIALS AND METHODS

An average of five animals from each of 21 species and subspecies of rodents were tested. Four of the six highland rodents were native to Peru: Akodon boliviensis, Phyllotis darwinii posticalis and feral Mus musculus from Morococha (14,900 ft or 4.5 km); and Calomys (=Hesperomys) ducilla, from near Puno (12,500 ft or 3.8 km). The Afghan pika (Ochotona rufescens) which ranges up to altitudes above 12,000 ft (3.6 km) (Puget, 1973), the Andean guinea pig (Cavia porcellus) and chinchilla, Chinchilla lanigera complete the list of highland species. Of these highland individuals only the 2 Akodon and 2 of the 5 Phyllotis were native born and they were held for more than 4 months near sea level before testing.

The fifteen lowland species and races represent ten other genera (Dicrostonyx groenlandicus, Clethrionomys rutilus, Peromyscus maniculatus, Citellus undulatus, Microtus oeconomus and pennsylvanicus, Octodon degus, Meriones unguiculatus, Baiomys taylori, Acomys cahirinus and Glaucomys volans) and three which were common to the high altitude group (Phyllotis darwinii limatus, Mus musculus and Calomys callosus). Because of the poor homeothermic behaviour shown by Acomys, Octodon and Calomys callosus at temperatures below the zone of thermoneutrality, they were measured

under lower metabolic demand than the rest of the species. Most individuals were young adults from breeding colonies which were maintained under standard conditions (Morrison, 1971). For original stocks, we are indebted to Dr. O. P. Pearson for Phyllotis, and C. ducilla, to Dr. K. M. Johnson for C. callosus, to Dr. A. Puget for Ochotona and to Dr. J. Hudson for Baiomys.

The general methodology follows Rosenmann & Morrison (1974a) except that most rodents were acclimated at 5 to 15°C for 4 to 6 weeks prior to the tests to ensure maintenance of normoxic body temperature at the temperature of the hypoxic tests. Oxygen consumption was measured in individually caged animals by means of a closed circuit automatic respirometer (Morrison, 1951).

The metabolic rate was recorded for about one hour at each of four to six levels of hypoxia which were produced by purging 5 to 6 volumes of the appropriate nitrogen-oxygen mixture through the metabolic chambers. Once established the levels were maintained as aliquots of O_2 consumed by the subject were automatically replaced. Values were confirmed with a paramagnetic oxygen meter. The lowest level of inspired P_{O_2} was 45 torr, equivalent to about 32,000 feet of altitude. Generally ambient temperatures (T_A), of 5 to 7°C elicited from 3 to 5 times the standard metabolism of mammals ($3.8\ W^{.73}$ $cc O_2/hr$, W in g) but in larger species such as Ochotona and Cavia a T_A of -5 to -9°C was required. Higher temperatures of 10-15°C provided an equivalent rate for smaller animals (Baiomys and Mus).

Maximum metabolic rates were obtained by exposing the animals to atmospheres in which He had been substituted for the N_2 at ambient temperatures from -5 to 10°C (Rosenmann & Morrison, 1974b). In addition, O_2 consumption was measured in most species at thermoneutrality (28 to 33°C) for 3 to 5 hours to determine the minimum resting rates in air (M_{min}). Respiratory frequency was measured at the same T_A by connecting a pressure transducer to the metabolic chambers and registering the output in a millivolt DC recorder. After a few hours the chambers were purged with a nitrogen-oxygen mixture of low P_{O_2}. Frequency values obtained during the initial adjustment period or during periods of activity in either atmosphere were discarded.

Three Acomys were acclimated to a P_{O_2} of 80 $\pm$ 8 torr at 21-23°C for 45 days utilizing an enclosure with silicone rubber membranes as described by Lange et al. (1968). Each week the hypoxic chamber was opened for about 1/2 hour for cleaning and feeding. It was then flushed with 10% O_2 to restore the hypoxic level. Blood was obtained by orbital bleeding directly into microhematocrit tubes.

The following symbols are used:

M_{st} = standard metabolic rate, 3.8 $W^{.73}$ $cc O_2/hr$ (W in g)

met = unit of metabolism representing multiples of M_{st} which allows direct comparison of animals of different weight

M = metabolic rate in hypoxia (met)

M_o = metabolic rate in normoxia (met)

M_{max} = maximum metabolism (normoxia)

M_{min} = minimum metabolism (thermal neutrality)

P = partial pressure of oxygen (torr)

P_c = critical pressure of oxygen (torr)

α = Hypoxic coefficient, the fractional reduction of M_o with change in P (%/torr)

RESULTS

The stepwise exposure to diminishing P_{O_2} allowed us to determine the minimal partial pressure of O_2 (P_c) at which the animals were able to maintain heat production. Below P_c the O_2 consumption fell in proportion to the decrease in P_{O_2} (Fig. 1). At temperatures below thermoneutrality the metabolic demand (M_o) varied between 2.8 and 4.6 met in different species but since the hypoxic coefficient α appears independent of M_o, values can be directly compared between species. In contrast, the critical pressure (P_c) does change with M_o, in the tundra vole (Microtus oeconomus) according to the function, $P_c = 73 + 13.2\ M_o$ (Rosenmann & Morrison, 1974a). Accordingly we have used this increment of 13 torr/met to adjust the P_c values obtained under different metabolic demand to the mean value of 3.8 met for the 21 taxa (Table 1). This is an approximation in that the value for this increment in P_c will undoubtedly vary somewhat in different species. However, the data of Segrem & Hart (1967) on Peromyscus leucopus yield a very similar value of 12 torr/met.

As presented in Table 1, both the hypoxic coefficient α and the critical pressure P_c were, in general, lower in the highland species.

Fig. 1 Metabolic reduction during hypoxia in 3 representative species. Symbols show mean, SE, SD and range.

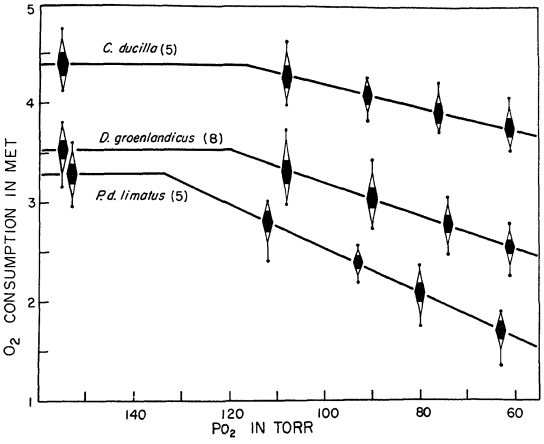


TABLE 1: Critical oxygen pressures (P_c) and metabolic coefficients (α) in rodents native to low and high elevations.

Species	Origin	Body Wt. g	N	M_o-3.8‡ met	P_c* torr	α %/torr
Phyllotis d. limatus	low	56	5	- 0.52	141	0.55
Octodon degus	low	181	2	- 0.96	139	0.73
Acomys cahirinus	low	49	3	- 0.90	131	0.83
Citellus undulatus	low	472	4	- 0.65	131	0.68
Glaucomys volans	low	67	4	- 0.65	128	0.80
Baiomys taylori	low	8	3	+ 0.47	127	0.84
Microtus oeconomus	low	36	6	+ 0.20	123	0.72
Meriones unguiculatus	low	48	6	+ 0.78	123	0.75
Dicrostonyx g. stevensoni	low	52	8	- 0.28	123	0.49
Clethrionomys rutilus	low	33	5	- 0.24	122	0.58
Cavia porcellus	high	481	5	- 0.69	117	0.49
Mus musculus (feral)	high	19	6	- 0.10	116	0.42
Mus musculus (white)	low	35	3	+ 0.38	116	0.89
Akodon boliviensis	high	26	2	- 0.18	113	0.30
Mus musculus (feral)	low	17	4	+ 0.48	112	0.91
Calomys callosus	low	48	6	+ 0.30	111	0.84
Peromyscus m. bairdi	low	21	7	+ 0.32	109	0.69
Calomys ducilla	high	18	5	+ 0.58	108	0.27
Phyllotis d. posticalis	high	78	6	- 0.88	107	0.65
Ochotona rufescens	high	176	4	+ 0.20	100	0.81
Microtus pennsylvanicus	low	29	7	+ 0.65	97	0.90

‡ Deviation from average M_o of 3.8 met

* Adjusted to 3.8 met following the function, $\Delta P_c = 13.2\Delta M$, for M. oeconomus

However the meadow vole, (M. pennsylvanicus) was a clear exception in showing the lowest P_c of any species (97 torr). Three other lowland species (P. maniculatus, C. callosus, Mus musculus) showed P_c values within the range of the highland rodents.

Figure 2 plots the fractional reduction in metabolism against P_{O_2} to compare the six highland species with ten of the lowland species. Values for species not plotted lie between 0.69 and 0.84 at 90 torr. Except for M. pennsylvanicus the highland species (heavy lines), show lower metabolic deficits in hypoxia than the lowland rodents (light lines). Closely related species from different altitudes showed marked differences in their response to the same hypoxic stimulus; thus C. callosus, P. d. limatus and M. musculus from low elevations (broken lines), showed 20 to 34% reduction in their normoxic metabolic rates at simulated 18,000 ft or 5.5 km; ($P_{O_2} \sim 80$ torr) as compared with only 9 to 15% reduction in the Andean species: C. ducilla, P. d. posticalis and M. musculus. The advantage of these species under hypoxia can be related to a lower P_c or a lower α. Thus in Phyllotis P_c was 96 torr ($\sim$13,000 ft or 3.9 km) in the Andean subspecies and 135 torr ($\sim$4,500 ft or 1.4 km) in the low altitude one. Contrasting with the large difference in P_c, α was only 0.1 %/torr lower in P. d. limatus. The reverse pattern was found in the three races of Mus musculus in which P_c only varied between 112 and 116 torr, while α in the lowland races (0.90 %/torr) was twice that in the Andean group (0.42 %/torr). In like manner both species of Calomys showed similar P_c values (108 and

Fig. 2 Metabolic reduction during hypoxia in 6 highland species (heavy lines), in 3 closely related lowland species (broken lines), and in 7 other lowland rodents (light lines). All values adjusted to a normothermic metabolic demand (M_o) of 3.8 met. Different species indicated by initials from Table 1.

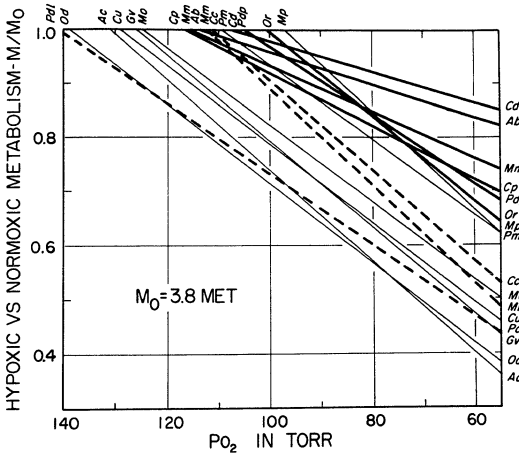
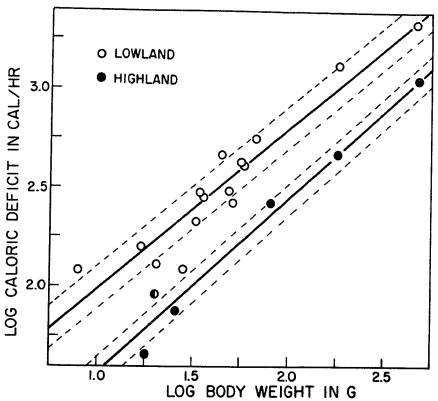


Fig. 3 Double logarithmic plot of the caloric deficit at P_{O_2} of 80 torr and initial metabolic demand of 3.8 met as a function of body weight. Regressions and SE of estimate indicated by solid and broken lines respectively. Half-closed circle represents the house mouse, a recent highland migrant.



111 torr) but α was three times higher in the low altitude species (0.84 vs 0.27 %/torr).

In Fig. 3 the caloric deficit at simulated 18,000 ft (5.5 km) and 3.8 met is plotted against body weight (log-log) and shows distinct regressions of:

$$\log (M_o - M) = 1.153 + 0.825 \log W \text{ (cal/hr,g)}$$

and $$\log (M_o - M) = 0.634 + 0.915 \log W \text{ (cal/hr,g)}$$

for the respective highland and lowland groups. The logarithmic increment between the two curves was 0.3-0.4 representing a 2-to 2 1/2-fold larger caloric deficit in the lowland.

Maximum metabolism under normoxic conditions (M_{max}) and also at P_{O_2} of 75 torr was measured in four lowland and three highland forms. The results of these measurements are shown in Table 2. The metabolic expansivity of both groups (difference between maximum and minimum values showed less variation when measured in normoxic conditions (4.0-6.7 met) than in hypoxia (1.9 to 5.3 met). The highest metabolic capability at P_{O_2} of 75 torr was shown by C. ducilla that was able to sustain 81% of its M_{max} in normal air. White and feral Mus from low altitude showed a 50% reduction at the same hypoxic level as compared with a 38% reduction in feral mice from high altitude. Although C. callosus showed less reduction in M_{max} in hypoxia (41%) than the white mice (50%), due to their low M_{max} the metabolic expansivity in hypoxia amounted to only 1.9 met as compared with 2.5-2.6 met in lowland Mus. Averaged, the lowland species at 75 torr showed a 54% reduction (47 - 60%)

TABLE 2: Maximum oxygen consumption and metabolic expansivities in normoxic and hypoxic atmospheres

Species	Origin	Maximum metabolism (M_{max})			Metabolic expansivity (M_{max}-M_b)		
		P_{O_2} 150 torr	P_{O_2} 75 torr	75/150 torr	P_{O_2}150 torr	P_{O_2} 75 torr	75/150 torr
		met	met	%	met	met	%
Microtus oeconomus	low	8.2(3)*	5.1(6)	62	6.6	3.5	53
Calomys callosus	low	5.0(6)	2.9(6)	58	4.0	1.9	48
Calomys ducilla	high	7.7(5)	6.3(6)	82	6.7	5.3	79
albino	low	7.4(5)	3.7(6)	50	6.3	2.5	40
Mus musculus feral	low	7.0(4)	3.6(4)	51	6.0	2.6	43
feral	high	7.7(6)	4.8(6)	62	6.7	3.8	57
Ochotona rufescens	high	6.6(6)	4.3(5)	65	5.2	2.9	56

* Number of animals in parentheses

in metabolic expansivity as compared with a 36% reduction (21 - 44%) in the highland group.

Respiratory frequencies measured at thermoneutral temperatures in normal air and during exposure to a P_{O_2} of 68 torr are shown in Table 3 together with predicted rates according to the weight function of Guyton (1947), as reference. Excluding the value for guinea pigs (+69%) the average deviations of our sixteen species from the expected frequency ranged from (+18% to -15%) and averaged less than 3%. The highland rodents increased their respiratory frequency in hypoxia by only 35% (13 - 57%) as compared to 81%, range (57 - 138%) in the lowland species. This response is illustrated in Fig. 4 in which the log respiratory frequency is plotted for hypoxic and normoxic conditions. Regression analysis gave the relations:

$$\log F_R 68 = 1.294 \log F_R 155 - 0.355 \text{ (lowland)}$$

and $$\log F_R 68 = 1.338 \log F_R 155 - 0.545 \text{ (highland)}$$

The log increment of 0.10 between the curves defines the 26% higher level of respiration in hypoxia in the lowland group.

As an exploratory effort to assess the effect of prolonged exposure to hypoxia on hypoxic resistance, 3 Acomys cahirinus were acclimated at a P_{O_2} of 80 $\pm$ 8 torr for 45 days at 22°C. Despite an increase in hematocrit from 48 $\pm$ 1% to 69 $\pm$ 2% there was no change in either P_c (130 vs 131 torr) or α (0.83 vs 0.83 %/torr). This may represent an overcompensation in which the advantage of increased oxygen capacity was offset by the disadvantage of increased viscosity since Smith and Crowell (1963) have

TABLE 3: Resting values of respiratory frequency in normoxic and hypoxic atmospheres.

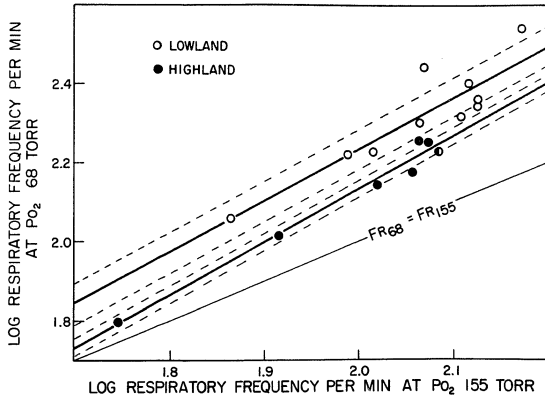
SPECIES	Body Wt	N	T_A	Respiratory frequency (breaths · min^{-1}) P_{O_2} 155 torr		P_{O_2} 68 torr	
				Predicted*	Observed**	Observed**	Increase
	g		°C	min^{-1}	min^{-1}	min^{-1}	%
Calomys ducilla‡	17	9	30	146	119±24 SD	179±39 SD	50
Peromyscus maniculatus b.	18	6	30	144	149±28	348±30	134
Mus musculus (feral)‡	19	5	28	143	121±19	170±18	41
Mus musculus (feral)	19	4	30	143	129±11	203±18	57
Clethrionomys rutilus	20	8	28	140	117±30	278±54	138
Microtus pennsylvanicus	26	6	28	130	134±21	220±22	64
Microtus oeconomus	27	8	28	129	131±28	249±60	90
Akodon boliviensis‡	32	1	28	124	116±10	182±15	57
Mus musculus (albino)	33	5	31	123	117±11	198±17	69
Acomys cahirinus	45	6	31	114	134±11	226±34	69
Calomys callosus	49	7	30	112	104±10	168±15	62
Dicrostonyx groenlandicus	57	10	28	108	98±17	165±26	68
Phyllotis d. posticalis‡	65	1	28	104	114±7	140±14	31
Ochotona rufescens‡	169	6	26	82	82±11	103±10	26
Rattus norvegicus	481	3	28	63	73±6	117±14	60
Cavia porcellus‡	513	3	26	62	105±10	138±24	31
Chinchilla lanigera‡	518	3	28	62	56±15	63±17	13

* $F_R = 295 \cdot W^{-0.25}$ (Guyton, 1947)

** average of 9.7 frequency values per individual

‡ highland species

Fig. 4 Double logarithmic plot of normoxic and hypoxic respiratory frequencies. Regressions and SE of estimate indicated by solid and broken lines respectively. Reference line indicates base for no change in frequency. Half-closed circle represents the house mouse, a recent highland migrant.



reported an optimal range of 37 to 54% hematocrit for survival of dogs in hypoxia.

DISCUSSION

In general our highland species as a group showed quantitative responses to hypoxia of a different magnitude than those shown by the lowland species. In Table 4 comparisons of the mean responses of both groups with respect to P_c, α, M/M_o, respiratory increase, and metabolic expansivity, indicate significant differences in all five measured parameters. Thus the average P_c for the highland rodents was 110 torr as compared to 122 torr in the lowland group. In terms of altitude the difference between groups was equivalent to 3,000 ft (10,000 vs 7,000 ft). The lowest P_c found in the highland species was in Ochotona, 3 torr above that in M. pennsylvanicus. The highest P_c observed was in Phyllotis d. limatus (141 torr), equivalent to an altitude of only 4,500 feet. In this species the limitation of O_2 consumption at such modest elevation was partly compensated by a low value for α.

At a metabolic demand level of 3.8 met, P_c ranged in our 21 mammals from 141 to 97 torr equivalent to the range of 3,300 to 13,000 ft in simulated altitude. This compares to a range of from 36 to 8 torr in 18 species of rodents measured under basal conditions by Hall (1966). Hall's highest P_c value was in Glaucomys volans, 10 torr above that in Meriones unguiculatus. Our P_c values for the same species indicates a similar difference, 5 torr higher in the gerbil than in the flying

TABLE 4: Mean hypoxic indices in highland and lowland groups

INDEX	HIGHLAND	LOWLAND	p
Critical O_2 pressure (P_c)			
Mean ± SD, torr	110±6.4 (6)*	122±11.7 (15)	<.05
Range, torr	100 - 117	97 - 141	
Equivalent altitude, Km	3.05	2.14	
Hypoxic coefficient (α)			
Mean ± SD, %/torr	0.49±0.21(6)	0.75±0.13(15)	<.01
Range, %/torr	0.27 - 0.81	0.49 - 0.90	
Metabolic reduction (to M/M_o)			
10,000 ft, 3.05 Km ± SD	0.99±0.001(6)	0.91±0.063(15)	<.01
18,000 ft, 5.5 Km ± SD	0.86±0.031(6)	0.69±0.077(15)	<.001
Respiratory increase‡ (breaths/min)			
Mean $R_{F_{68}}$ vs $R_{F_{155}}$, %±SD	35±15(7)	81±30(10)	<.01
Range, %	12.5 - 56.8	57.4 - 137.6	
Metabolic expansivity ratio			
M_{max} - M_b (75/155 torr)	0.64(3)	0.46(4)	<.05
Range	0.56 - 0.79	0.40 - 0.53	

* Number of taxa in parentheses

‡ Excluding *Cavia*, the normoxic frequency was identical in lowland and highland forms (e.g., 122 vs 118/min for animals of 17-65 g)

squirrel. A correlation of body size with the ability to extract oxygen at low tensions has also been proposed in the same study but regression analysis in our 20 rodents (plus pika) showed the relation

$$\log P_c = 2.036 + 0.0214 \log W \text{ (torr,g)},$$

indicating substantial independence of P_c with respect to body size ($r = 0.226$, $P>0.1$). Representing lethal levels (M_0 = ca 1 met), Hall's values for P_c are not easily compared to our range (M_0 = 2.8-4.6 met) but they do accentuate the importance of the level of metabolic demand in defining P_c. The modifying effect of the metabolic demand on P_c and consequently on the animals response to hypoxia was also shown by Segrem & Hart (1967) in Peromyscus leucopus.

The hypoxic coefficient, α, (related to the "biotic efficiency" of Chevillard, 1966) represents a degree of metabolic impairment below P_c. Thus our mean value of α in the lowland rodents (0.75 %/torr), indicates a larger hypoxic involvement than in the highland species (0.49 %/torr). Similarly, Bullard & Kollias (1966) reported a larger metabolic depression in lowland Citellus than in highland ones exposed to the same degree of hypoxia and T_A. Regression analysis of α on body weight gave the equation

$$\alpha = 0.677 - 0.0021 \log W \text{ (torr}^{-1}\text{, g)}$$

for our two groups of rodents ($r = -0.005$, $P > 0.1$). Thus α as well as P_c seemed to be independent of body size within our range of 8 to 480 grams.

The P_c value observed in Peromyscus maniculatus was near the average for the highland group and it is of interest that this species

ranges up to 14,000 ft (Hock, 1961). Similarly, although M. pennsylvanicus may not occur at high alitude, other Microtus (M. mordax, M. montanus) have been reported at 12,000 ft (Bole, 1938). The low sensitivity to hypoxia in M. pennsylvanicus may show its ability to inhabit similar elevations but its actual distribution may be restricted by other factors such as moisture (Lewin, 1968; Heisinger et al., 1973). Monge & Monge (1966) noted that although hypoxia is the most significant factor in high altitude biology it would be misleading to believe that it acts independently of other factors such as temperature, dryness and radiation.

Because our subject rodents present a wide range of types varying in many attributes it is difficult to associate specific features with degrees or types of hypoxic resistance. However, comparisons between closely related forms may allow some conclusions (Table 5). Thus, of the two subspecies of Phyllotis, the highland P. d. posticalis showed a much lower P_c (107 vs 141 torr) while α was actually a little higher (0.65 vs 0.55 %/torr). This change in P_c correlates with the greater oxygen affinity shown by the hemoglobin of P. d. posticalis (1.8 x P.d.l.) (Morrison, 1974). Myoglobin levels (Reynafarje & Morrison, 1962), heart and respiration rates (Morrison & Elsner, 1962) also differ between highland and lowland subspecies of Phyllotis.

In a like manner we can compare the races of Mus musculus from Morococha and from Arkansas which are similar in P_c (116 vs 112 torr) but very different in α (0.42 vs 0.91 %/torr). Here, the similarity in P_c correlates with that in oxygen affinity of the hemoglobins which

TABLE 5: Comparison of hypoxic indices in related rodents with the P_{50} of their hemoglobins

	ORIGIN	P_{50}*	P_c	α
Phyllotis d. posticalis	high	14	107	0.65
Phyllotis d. limatus	low	25	141	0.55
Mus musculus (feral)	high	18	116	0.42
Mus musculus (feral)	low	--	112	0.91
Mus musculus (white)	low	18	116	0.89
Calomys ducilla	high	17	108	0.27
Calomys callosus	low	--	111	0.84

* For 3 g/l solutions at pH 7.0, $\Gamma/2$ = 0.15 and 25°C, (Morrison, 1974)

is the same in these two races. The two species of Calomys showed the same pattern as Mus, similar in P_c (108 vs 111 torr) and divergent in α (0.27 vs 0.84 %/torr). There is no reference data on hemoglobin from C. callosus, but that of C. ducilla with the lowest O_2 affinity of some dozen highland species was close to that of Mus.

Although among lowland rodents other Citellus have shown higher survival in severe hypoxia or a greater improvement in functional capacity (Hiestand et al., 1950; McLaughlin & Meints, 1972), our arctic ground squirrels showed no special advantage. The metabolic expansivity ("scope for activity" of Hart & Jansky, 1963) under hypoxia was clearly higher in C. ducilla (5.3 met) than the other species tested. A lower value (3.8 met), but still higher than in lowland Mus (2.6 met), was found for Andean house mice. The difference may relate to the much more recent introduction of the house mouse to high altitude at Morococha as compared to the indigenous C. ducilla. At a similar P_{O_2}, Segrem & Hart (1967) found a reduction to about 60% of M_{max} in Peromyscus leucopus. Similarly, our values for C. callosus, M. oeconomus and feral Mus range from 50 to 62% of M_{max}.

Normoxic respiratory frequency followed within 1 SD the predicted values for mammals of different body sizes: $F_R = 295\ W^{-0.25}$ (Guyton, 1947). Regression analysis for our 16 species and subspecies gave a similar relation, $F_R = 248\ W^{-0.21}$ ($r = -0.923$, $P<0.001$), the slightly lower weight exponent almost identical to that of -0.20 calculated by Mead (1960) on the basis of minimal pressure amplitude. Although

the respiratory rate is not a direct measure of lung ventilation its increase at low P_{O_2} could be a measure of the response to hypoxic stress. Thus the highland species showed lower breathing rates under hypoxia than the lowland rodents, corroborating the low metabolic impairment shown by this group. M. pennsylvanicus, P. maniculatus and D. groenlandicus were the most hypoxic-resistant lowland species, as judged between 100 and 70 torr. Nevertheless their increase in respiration during normothermic hypoxia was 2 to 4 times that of the highland species, suggesting a larger compensatory effort or a higher sensitivity to hypoxia.

In all these comparisons which show differences between highland and lowland species it should be kept in mind that our highland subjects were largely born and raised at sea level. Thus the observed differences were genetic and not the result of acclimatizational effects during maturation which might well accentuate these differences in comparisons of highland and lowland forms resident in their respective environments.

SUMMARY

The metabolic response to hypoxia at moderate O_2 demand was measured in 7 highland and 15 lowland species and races of small mammals. The critical ambient P_{O_2} for reducing O_2 uptake was lower in the highland group (110 vs 122 torr). Below this P_{O_2} the further metabolic reduction was also lower in the highland group (0.49 vs 0.75 %/torr). The metabolic expansivity at 75 torr O_2 was larger in the 3 highland species tested than in 4 lowland ones (4.0 vs 2.6 met), representing a reduction in the normoxic maximums of 36 vs 54%. In normoxia the respiratory frequency for both highland and lowland species followed the function $248\ W^{-0.21}$. The hypoxic increase of respiratory frequency at a P_{O_2} of 68 torr was lower in the highland forms (35 vs 81%).

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CHAPTER 3

MAXIMUM OXYGEN CONSUMPTION AND HEAT LOSS FACILITATION IN SMALL HOMEOTHERMS BY He-O_2

INTRODUCTION

Although animals in nature spend the majority of their time resting or at low levels of activity, some functions critical for survival such as fighting, escaping or predation greatly increase the energy demand. The maximum capability to increase metabolism is the main factor in limiting the intensity of such physical activity and, as well, sets the limit for cold tolerance. Evaluations of maximum oxygen uptake (M_{max}) in mammals have been made by cold exposure in air or water, by exercise on treadmills, or by swimming, or by a combination of these. These methods may require extended training sessions for running (as long as a month), cutting off the tail, and elimination of inept performers (32). Another serious reservation is that these procedures may directly modify the character under study. Exposures to sub-freezing temperatures risks peripheral cold injury or lethal hypothermia (23). Removal of the natural insulation can increase metabolism greatly but, again, is traumatic and results in irreversible change.

To avoid such extreme cold exposure and the injuries reported during exercise (32,16), we have developed the use of a helium-oxygen atmosphere to raise the heat output of small homeotherms to maximum levels at more modest ambient temperatures (T_A). The effect of He-O_2 in increasing metabolism has been known for some time (3,36) and although once in some question its action is now generally ascribed to the increased thermal conductance of the gas (5,25,34). Since the insulative quality of fur or feathers depends on the slow transfer of heat through the enclosed air spaces, the substitution of a four-times more conductive

medium such as 80% He-20% O_2 (26) should greatly facilitate heat transfer. However, the actual metabolic increases reported in small mammals are so much more modest, e.g., < 25% in rats (34) or < 50% in mice (3,10) that the exact nature of the effect is still very much in question.

METHODS

Tests were conducted on two laboratory species, the white mouse and the white rat, previously assessed for M_{max} by various other procedures. Three further strains of Mus musculus, a hairless mutant (HRS/J) and feral house mice from 15,000 feet at Morococha, Peru, and from Arkansas were also compared. In addition, measurements were made on wild species native to different habitats, arctic (Microtus oeconomus from Fairbanks), alpine (Calomys ducilla from 13,000 ft near Puno, Peru) and subtropical (Calomys callosus from San Joaquim, Beni Province, Bolivia) and the pygmy mouse Baiomys taylorii. The final species was a small arctic bird, the redpoll (Acanthis flammea) also native to Fairbanks. We are indebted to Dr. Oliver P. Pearson, and Dr. Karl M. Johnson for the respective Calomys, to Dr. Hermann Pohl for the redpolls and to Dr. John Sealander for the lowland Mus. These rodents were maintained in our animal facility at a neutral T_A of 20-24°C. Three to eight individuals of each type were tested at 735 to 750 Torr.

O_2 consumption was measured in a closed circuit manometric respirometer (28). Since the oxygen consumed by the animal is replaced by

successive aliquots, the inert component maintains its concentration as established. The metabolic chambers of stainless steel were submerged in a thermoregulated water-glycol bath. After a variable length of time (1 to 3 hours) in which the metabolic rate in air was measured, the chambers were flushed with 5 to 6 times their volume of an 80% He- 20% O_2 mixture from a proportioning gas mixing pump or from a gas tank. Use of the gas tanks with premixed He-O_2 reduced the purging time to only 2 minutes as compared with 7 to 9 minutes with the mixing pumps. To avoid temperature changes, the He-O_2 mixture was admitted through a submerged copper coil.

Food as apple, carrot and sunflower seed was generally available during the tests. Regardless of the duration and temperature of the metabolic measurements, the animals were tested about 4 times a week and generally 2 days were allowed between consecutive tests at high submaximal rates.

RESULTS

Immediately after the substitution of He-O_2 for air, O_2 consumption increased well above the resting levels in air, as shown in Figure 1 for M. oeconomus. Of interest also is the suppression in He-O_2 of the metabolic cycles reflecting changes in activity or posture, an effect also seen at lower T_A in air. These relations are shown in Fig. 2 for a series of T_A between 26 and 7°C again for M. oeconomus. The respective values in air and in He-O_2 lie along two straight lines which extrapolate

Fig. 1 Representative experiment showing the effect of He-O_2 on oxygen consumption at 15°C in M. oeconomus

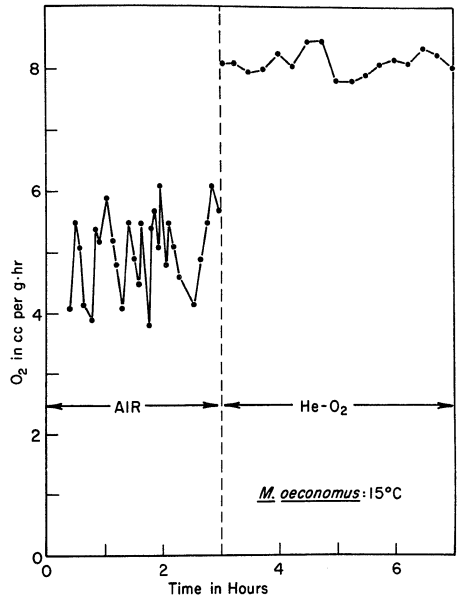
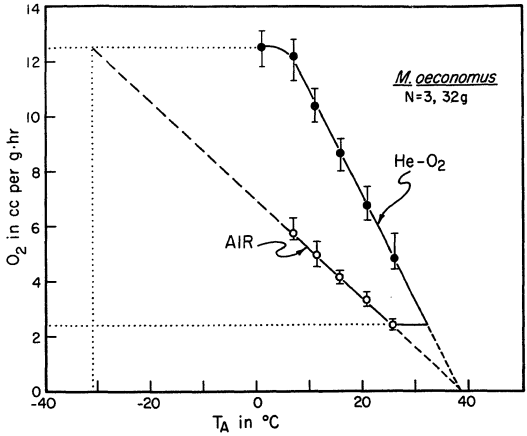


Fig. 2 Oxygen consumption of M. oeconomus in air (open circles) and in He-O_2 (solid circles) measured at different temperatures. Upper and lower dotted lines indicate observed M_{max} and M_{min} respectively. Vertical dotted line from intercept M_{max} and M_{air} indicate extrapolated air temperature for M_{max}. Vertical bars show range of values.



to the T_B at 39°C to fit the relations:

$$M = C(T_B - T_A) = C\Delta T \text{ and } M = C_{He}\Delta T$$

The constant C usually designated as the (minimum) conductance actually includes a component of evaporative/respiratory heat loss but at these T_A below thermal neutrality this represents a constant (small fraction of the thermal loss through the surface) a cost of "chemical" thermoregulation. Accordingly, C is a measure of the overall facility for heat loss from the body and will reflect changes in the properties of the insulative layers. In this example the substitution of He-O_2 increased conductance from 0.178 to 0.377 $cc O_2$ $(g \cdot hr \cdot °C)^{-1}$, a factor of 2.12 ($C_{He}/C$).

At temperatures below the thermoneutral zone heat production more than doubled in He-O_2 until at about 6°C (in this species) a limit was reached indicating maximum metabolic capability for temperature regulation (M_{max}). In fact, at 1°C in He-O_2 the maximum heat production could only be maintained for approximately 5 minutes and longer exposure under these conditions resulted in decreased O_2 consumption and hypothermia. Exposure to lower temperatures in He-O_2 did not seem to modify their maximum response but the highest rates were held for a shorter time. The quotient, M_{max}/C, provides an estimate of the maximum temperature differential tolerable by the animal, in this case -70°C. This has been shown graphically by extrapolation of $M - C\Delta T$ to the value M_{max} = 12.5 $cc O_2$ $(g \cdot hr)^{-1}$ at -31°C. It may be noted that we have observed limiting values of cold tolerance of this species (neutral acclimation) at -25°C to -30°C (unpublished observations).

A somewhat lower ratio was observed in white rats (Fig. 3). Maximum rates in He-O_2 were attained within 6 to 10 minutes at -3°C. Exposure at -10°C in He-O_2 resulted in reduced O_2 consumption and hypothermia.

The effects of He-O_2 on highland house mice and white mice are shown in Fig. 4. The ratio C_{He}/C was 2.3 in the house mice as compared to 2.1 in white mice and M_{max} was 24% higher, 13.8 vs. 11.1 $cc O_2\ (g \cdot hr)^{-1}$. In lowland house mice the M_{max} was intermediate at 12.3 $cc O_2 (g \cdot hr)^{-1}$.

To gain insight into the relation between the surface insulation and the effects of He-O_2, hairless mice were also tested (Fig. 5). The ratio of the thermal conductance in air of hairless vs. normal mice was 1.9, close to a previously reported ratio of 2.2 for these strains (29). However in He-O_2, the conductance ratio hairless vs. normal was only 1.3 due to the much smaller response of the hairless mice to helium (C_{He}/C = 1.40). M_{max} in hairless mice was 13% higher than in the normal white mice (12.5 vs. 11.1 $cc O_2\ (g \cdot hr)^{-1}$) but still 10% lower than in the feral mice. Similarly, the metabolic expansivity of the hairless mice (10.5 $cc O_2\ (g \cdot hr)^{-1}$) lay between those of the two other strains. M_{max}/M_{min} ratios were 6.3 in both the white and the hairless mice as compared with 7.2 - 7.3 in both feral groups.

Results of He-O_2 exposure in Calomys ducilla, a highland species, and Calomys callosus, a tropical species, are shown in Fig. 6. M_{max} of 14 $cc O_2\ (g \cdot hr)^{-1}$ was found in the 16-gram C. ducilla and 6.8 cc O_2

Fig. 3 Oxygen consumption of white rats in air (open circles) and in He-O_2 (solid circles) as a function of T_A.

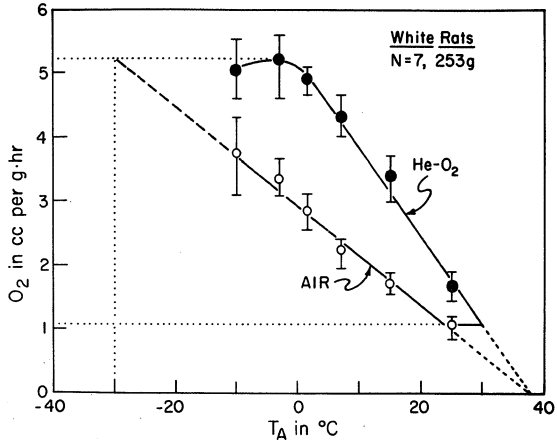


Fig. 4 Oxygen consumption of feral house mice (circles) and of white mice (squares) in air (open symbols) and in He-O_2 (solid symbols) as a function of T_A.

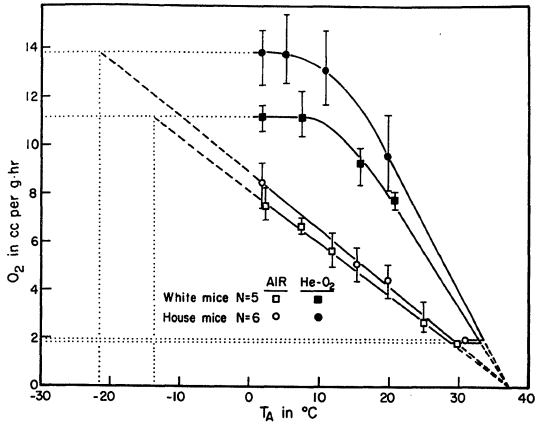


Fig. 5 Oxygen consumption of hairless mice in air (open symbols) and in He-O_2 (solid symbols) as a function of T_A. Reference curves compare function for normal mice.

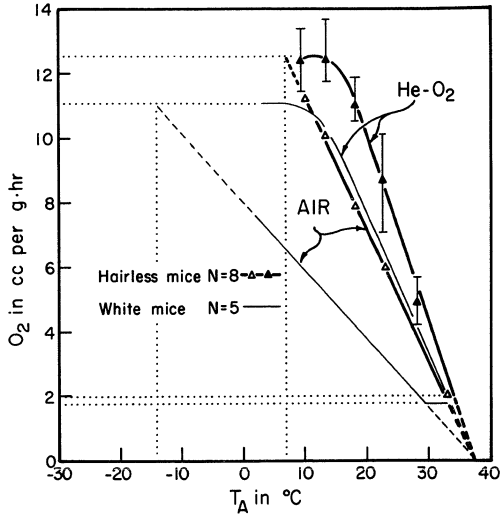
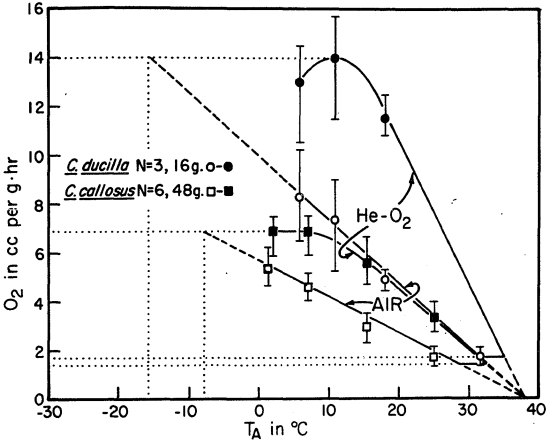


Fig. 6 Oxygen consumption of Calomys ducilla (circles) and of C. callosus (squares) in air (open symbols) and in He-O_2 (solid symbols) as a function of T_A.



$(g \cdot hr)^{-1}$ in the three times larger C. callosus.

Redpolls (Acanthis flammea) were treated in a similar way to the rodents with the exception of a wooden perch in the cages and an aluminum cover to maintain a dark environment and so diminish spontaneous activity. O_2 consumption values in air and in He-O_2 are shown in Figure 7. The highest ratio, $C_{He}/C = 2.6$, was observed in the redpoll and an M_{max} of 21.8 $cc O_2$ $(g \cdot hr)^{-1}$ was elicited at -5°C.

Table 1 summarizes the thermogenetic effects of He-O_2 in the experimental species, the temperatures at which M_{max} was elicited in He-O_2, the extrapolated air temperatures for these rates and the thermal conductance values in relation to the He-O_2 atmosphere and to the animal's surface area. In general, M_{max} in the rodent species was obtained in He-O_2 at 13 to 30°C warmer temperatures than expected in air, but in the hairless mice the difference was only 7°C. In contrast, a 70°C warmer temperature for M_{max} elicitation in He-O_2 was estimated for the redpolls.

DISCUSSION

A comparison of our data on M_{max} of mice, rats, and of the pygmy mouse with reported values for the same species but obtained with different methodologies is shown in Table 2. Our values for white mice are equal or higher than reported figures from mice kept at neutral or warm temperatures but are 8% lower than from cold acclimated mice (12). Similarly in white rats, M_{max} values in He-O_2 are the same or higher than reported capabilities in normal rats and

Fig. 7 Oxygen consumption of redpolls in air (open circles) and in He-O_2 (solid circles) as a function of T_A. Continuous line down to -30°C indicate O_2 consumption in summer (M - 8.24 - 0.18 T) after West (35). Symbols at -50°C indicate O_2 consumption values in spring (1) and summer (2), after Pohl and West (33).

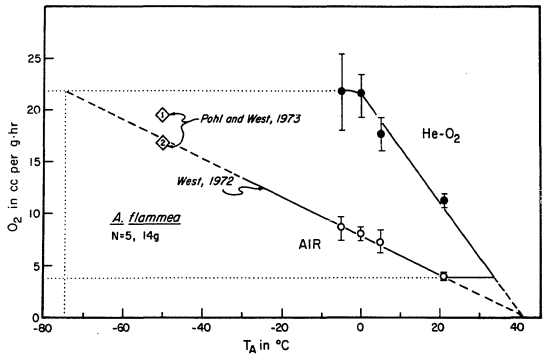


TABLE 1. Metabolic ratios and conductance factors in He-O_2 and air.

SPECIES	Wt.	N	$\frac{M_{max}}{M_{min}}$	$\frac{M_{max}}{M_{st}}$ †	T_A for M_{max} He-O_2	T_A for M_{max} Air	C $\frac{cc O_2}{g \cdot hr \cdot °C}$	C^{He}/C	$\frac{1(\ddagger)}{C/A}$ $\frac{cm^2 \cdot hr \cdot °C}{cc O_2}$
	(g)				°C	°C			
Rattus norvegicus (albino)	253	7	4.9	6.1	- 3	-30	.078	1.8	16.2
Calomys callosus	48	6	4.6	5.1	7	- 8	.132	1.9	16.7
Microtus oeconomus	32	3	5.2	8.4	1	-31	.178	2.1	14.2
Mus musculus (albino)	30	5	6.3	7.3	7	-14	.216	2.1	11.9
hairless	21	8	6.3	7.5	14	7	.575	1.4	5.0
wild, highland	17	6	7.3	7.8	5	-22	.236	2.3	13.1
wild, lowland	17	4	7.2	7.0	10	-12	.247	2.0	12.6
Calomys ducilla	16	3	8.2	7.8	10	-16	.254	2.3	12.5
Acanthys flammea	14	5	5.6	5.9	- 5	-75	.197	2.6	16.8
Baiomys taylorii*	7	6	4.3	5.5	23	10	.455	1.8	8.7

* Old Subjects, 18-28 months.

† M_{st} taken as 3.8 $W^{.73}$ for mammals and 7.54 $W^{.724}$ ($cc O_2/hr,g$) for passerines (24).

‡ Represents insulation per unit of surface area taken as 8 $W^{2/3}$.

TABLE 2. Comparison of maximum metabolism and M_{max}/M_{min} ratios obtained with different techniques

SPECIES	Wt.	Acclim.†	M_{max}	$\frac{M_{max}}{M_{min}}$	Reference and Technique
	(g)		$cc O_2/g \cdot hr$		
Mus musculus					
albino	18.5	--	10.75	3.5	(8) Cold water wetting
	26.5	--	10.10	5.2	(13) Run 6m/min at 2°C
	26.7	c	12.15	6.5	(12) Run 5m/min at -10°C
	33.0	c	10.50	--	(32) Run 23m/min at -10°C
	34.0	w	9.30	--	(32) Run 19m/min at -3°C
	33.4	c	7.40	4.2	(18) Noradrenaline, 1.7 mg/kg
	29.5	n	11.10	6.3	(‡) He-O_2 at 7°C
hairless	21.0	n	12.50	6.3	(‡) He-O_2 at 14°C
wild, highland	17.3	n	13.80	7.3	(‡) He-O_2 at 2 to 5°C
wild, lowland	17.0	n	12.30	7.2	(‡) He-O_2 at 10°C
Rattus norvegicus					
albino	380	w	3.20	2.7	(19) Cold exposure at -35°C
	385	--	2.76	2.7	(4) Cold exposure down to -33°C
	290	--	4.85	3.5	(27) Swim with 2% load
	115	--	4.81	3.6	(8) Cold water wetting
	300*	w	3.20	2.7	(17) Run at 0°C or rest at -35°C
	300*	c	5.05	4.2	(17) Run at -30°C or rest at -45°C
	286	w	4.90	3.1	(32) Run 35m/min at 6°C
	334	c	5.40	--	(32) Run 27-37m/min at 6 to -3°C
	253	n	5.20	4.9	(‡) He-O_2 at -3°C
	371	c	(∿5.3)	(∿5.0)	(22,23) (Cytochrome oxidase activity)
Baiomys taylorii	7.3	n	10.40	5.3	(20) Exposure at 7 to 15°C
	6.9	n	12.30	4.3	(‡) He-O_2 at 23°C

† acclimation: cold, warm, or neutral T_A
* assumed
‡ this study

only 4% lower than cold acclimated ones (32). In Baiomys, 18% higher M_{max} was obtained in He-O_2 than the maximum values reported with cold exposure (20).

Published data for the rest of our wild species seem to be unavailable, but some values have been reported in related species. In 18-gram Microtus arvalis a ratio M_{max}/M_{min} 5.5 has been reported in treadmill studies (21). Our value for 32-gram Microtus oeconomus was 5.2.

In summer birds, exposure of house sparrows to -30 to -65°C resulted in M_{max} of 15 $cc O_2$ $(g \cdot hr)^{-1}$ and M_{max}/M_{min} of 3.3 (14). In the goldfinch, exposure to 0°C after removing the feathers gave M_{max} of 18.0 $cc O_2$ $(g \cdot hr)^{-1}$ and M_{max}/M_{min} of 4.2 (8). By comparison M_{max} of summer redpolls in He-O_2 was 21.8 $cc O_2$ $(g \cdot hr)^{-1}$ with a M_{max}/M_{min} ratio of 5.6.

In previous studies of white mice and rats considerably smaller effects of He have been reported (34,36). Examination shows that those measurements, sometimes on groups of animals rather than individuals, gave reference values in air which clearly include a considerable activity component which is then suppressed under the cooling influence of the He (compare Fig. 1). Nor will measurements made within the thermoneutral zone show as large an increase in metabolism with He-O_2.

Free/forced convection and radiation present major routes for heat loss from bare skin with little or no involvement of simple conduction. Convective heat transfer in He-O_2 as compared with air has been described by Epperson et al. (1966), in relation to the ratios

of their thermal conductivities, densities, specific heats, and viscosities, together representing a ratio of 2.1. A somewhat larger value has been calculated for conductive-convective effects (7). By contrast to bare skin, conductive heat transfer should be of greater importance through fur (11), and a facilitation of 4-fold is indicated (air → He-O_2). However, it is not easy to assess the effective thickness of a fur, which will depend on the density, length, erection angle and uniformity of the fibers which modify convective mixing in the outer layers. Neither is it easy to assess the amount of radiant loss either from the outer layer of fibers or from more thinly covered areas as on the face, feet or tail. Other factors not directly influenced by He are surface evaporation and tissue insulation, with involvement of the latter suggested by lower skin temperatures in He-O_2 (34). Although the increase in conductance in the He-O_2 mixture cannot define all these factors quantitatively it should give us some measure of the relative importance of conduction (f = 2) radiation, tissue insulation and surface evaporation (f = 1).

As noted in Table 1, C_{He}/C ranged from 1.4 in the hairless mouse to 2.6 in the redpoll. Since a thick layer of fur or feathers will emphasize conductive transfer as compared to other modes C_{He}/C ratio should vary directly with the insulation. That such is the case may be seen in Fig. 8 for species from 7 to 32g. The larger C. callosus and white rat, however, do not conform to this pattern perhaps because of greater reliance on tissue insulation. Similarly, induction of hypothermia in He-O_2 has been also reported to be related to body

Fig. 8 Metabolic effects of He-O_2 (C_{He}/C) in different species vs. insulation per unit area, calculated as the reciprocal of the conductance per unit area: C/A with $A = 8\ W^{2/3}$. Mus musculus indicated in square symbols: hairless; albino; wild; lowland; wild, highland. The rest of the species indicated by initials in circles.

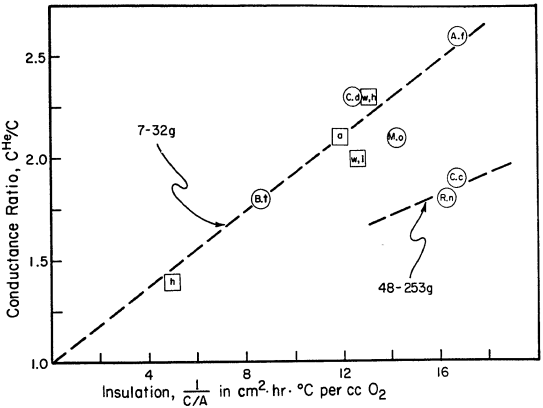
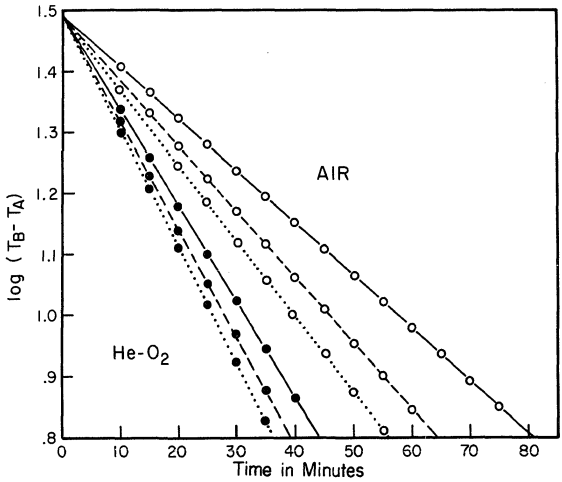


Fig. 9 Cooling curves for individual Microtus oeconomus in He-O_2 (left) and air (right). Solid lines, intact fur; dashed lines, fur clipped to about 1 mm; dotted line, fur totally removed.



size in rats of 100 to over 200 g (31). The relation between conductance and He facilitation was confirmed experimentally by observations of cooling rates in air and He-O_2 in preserved Microtus oeconomus with different degrees of insulation (Fig. 9). These results show conductance ratios of 1.83 when the fur was intact; 1.62 when the fur was clipped to 1 mm, and 1.52 when the fur was totally removed.

As heat dissipation is seen to be much more affected by insulation in air than in He-O_2 (>3 times), a simple way of describing the metabolic effects of helium in different species is in terms of the functional removal of the surface insulation regardless of its degree or quality, the metabolic increase then being proportional to the magnitude of the insulation so "removed". Thus well insulated animals, sensitive to small changes of skin temperature (15), would show a larger response.

Among the many applications of inert gases, He-O_2 atmospheres have been used for therapeutic reasons (2,9), also for prevention of nitrogen narcosis in divers (1) and in recent years for induction of hypothermia in small mammals (6,30,31). The elicitation of maximum O_2 consumption in small homeotherms may prove to be another practical application. The rapidly attained M_{max} values (3 to 10 minutes), the simplicity of operation, the avoidance of extreme cold temperatures and of treadmills and training tests are definite advantages of the present technique over the current conventional methods.

SUMMARY

The high thermal conductance of an 80% He-20% O_2 atmosphere was used to elicit maximum metabolism (M_{max}) in moderate cold in species ranging from 7-g pygmy mice (Baiomys taylorii) to 250-g white rats, including redpolls (Acanthis flammea), two vesper mice (Calomys ducilla, C. callosus), tundra voles (Microtus oeconomus), and four strains of Mus musculus. Values slightly exceeded those in similar animals using other methods to confirm the lower metabolic ratio (M_{max}/M_{min}) in rodents (4-8 x). Submaximal values at higher temperatures defined thermal conductance in He-O_2 and air. In different species the ratios of these conductances ranged from 1.4 to 2.6, differences which relate to the extent and quality of the respective insulation. M_{max} was obtained at 13 to 70°C warmer in He-O_2 than required in air for the same metabolic effort. Avoidance of low temperature technology and freezing injury, elimination of treadmills and training in running, prompt attainment of M_{max} (3 to 10 minutes after He-O_2 exposure) and obviation of shaving or wetting procedures are advantages of the present technique.

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CHAPTER 4

PHYSIOLOGICAL CHARACTERISTICS OF THE ALARM REACTION IN THE DEER MOUSE *PEROMYSCUS MANICULATUS BAIRDI*

INTRODUCTION

Hypothermia in small homeotherms can result from cold exposure when augmented heat loss exceeds the animal's metabolism. It can also occur when heat production is restricted by lowering pO_2, closely confining the animal, or by binding the thorax or hind limbs (Bartlett et al., 1953). In somewhat less artificial situations hypothermia may develop when animals are presented with a strange environment (Grant, 1950) and emotional hypothermia in rats has also been suggested (Bartlett, et al., 1954).

After restraint by humans or by predators some species show an "immobility response", also called "animal hypnosis" or "tonic immobility" which is characterized by the animal remaining quiet and unresponsive (Borchelt and Ratner, 1973). In the quail a "trance state" has been described by Doty (1969), and durations of immobility ranging from a fraction of a minute up to several hours are reported for other species including domestic fowl and reptiles (Ratner, 1967; Prestrude and Crawford, 1970).

Like tonic immobility, the freezing response is characterized by the animal remaining motionless when stimulated not by handling but by observing a strange object, approaching predators, or hearing calls from other animals. Behavioral scientists thus distinguish the initial freezing produced by the approach of a predator from tonic immobility after the predator seizes or touches the animal, but both states appear not to have been characterized other than by lack of movement. Stokes (1967), has

described the freezing response of quails after a predator's appearance in the sky as one form of a more general alarm reaction.

In laboratory experiments Hart (1953), described some Peromyscus maniculatus gracilis as hypothermic, lethargic, and not attempting to avoid capture or to escape but no explanation was given for this behavior. Our observations in the same species have indicated a similar pattern occurring mainly when deer mice were disturbed or alarmed by the presence of the observer above the animals or by objects moving above them. In the present study we have undertaken to systematically characterize some basic physiological aspects of this behavioral response.

METHODS

The experimental animals, Peromyscus maniculatus bairdii, originally from Madison, Wisconsin had been kept for several generations in our animal colony. They were maintained at 20°C with a 16-hour daily light cycle. During the measurements they were individually caged in glass covered steel chambers of 10 to 17 times the animal's volume which allowed for about 3 lengths movement. O_2 consumption was measured by connecting the chambers to a closed circuit automatic respirometer (Morrison, 1951). Body temperature (T_B) was measured after the experiments by a YSI telethermometer, or was continuously monitored using intraperitoneally implanted temperature transmitters.

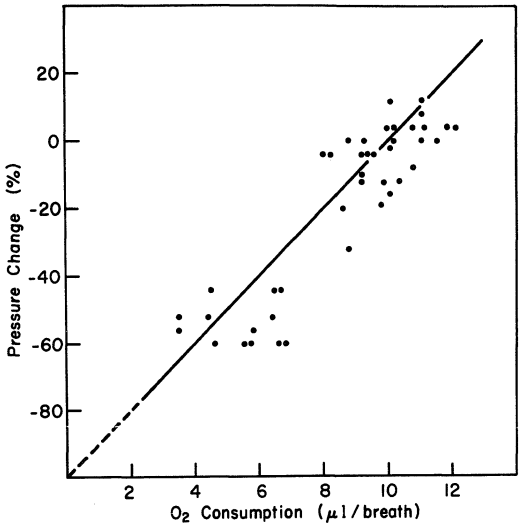
Pulse rates were obtained through parallel electrodes of copper wire which constituted the floor of the animal cage and were brushed with

conductor paste before the experiments. Two multiple switches were used to relocate the electrical signal when the animal changed position. Reported difficulties of hard-wire recording leads attached to a small mammal (Morhardt, 1970) were avoided in this way.

Respiratory frequency and estimates of tidal volume change were made using a pressure transducer adapted from an aircraft rate-of-climb meter and connected to the metabolic chamber and to a 2-channel DC/AC portable Gilson recorder which also received the electro-cardiographic input. Within our experimental range of respiratory frequencies, the amplitude of the signal was proportional to the pressures developed by pumping in and out different volumes of air with a 0.5 cc syringe, but this model does not correspond to the experimental situation in which the expandable element (chest) lies within the chamber. However, in animal experiments in which O_2 consumption, respiratory frequency and pressure amplitude were all measured, the volume of O_2 used per breath was directly proportional to the change in pressure as shown in Fig. 1. Accordingly, we do feel that this amplitude is a reasonable measure of the tidal respiratory volume.

The metabolic chambers were submerged in a thermostated water bath 20 cm below a suspended 38 cm diameter aluminum disc. By removing a 135° segment of the disc, a moving shadow was then projected as the disc rotated between the experimental animals and a light source. Controlled speeds of 5 to 500 RPM were easily obtained but because some vibration and noise were generated above 200 RPM most observations were conducted between 8

Fig. 1 Correlation of O_2 consumption per breath with recorded changes in pressure in the metabolic chamber.



and 100 RPM. During the control and undisturbed periods the disc was positioned to provide full illumination of the chamber.

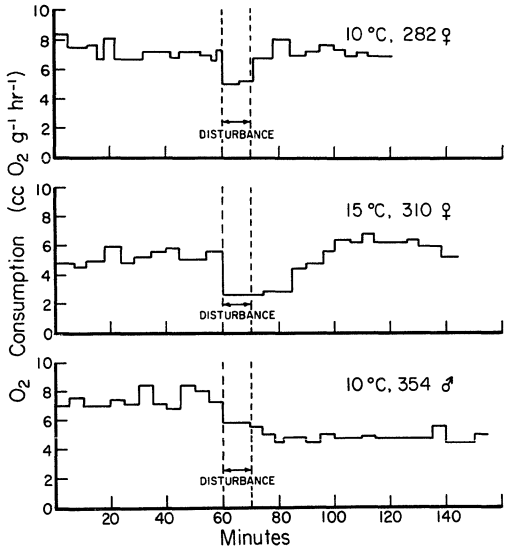
In different experiments, control periods of not less than one hour were provided to familiarize the animals with the system before measurements were initiated. Torpor due to shortage of food as reported to occur in this and other species of Peromyscus (Morhardt, 1970b) was avoided by allowing free access to sunflower seeds and oats in experiments of longer than 2 hours duration.

RESULTS

Rotating the disc at 30 RPM elicited an immediate reduction in O_2 consumption which was maintained for at least 8 minutes. Similar results were obtained by the horizontal movement of objects of different shape above the chamber or by the hand of the observer. After a series of metabolic runs on different animals, it became evident that although all deer mice showed some response, the length and intensity of the metabolic depression was quite variable in different individuals although rather constant in the same animals. Figure 2 illustrates three typical metabolic responses to alarm in different animals: (a) a short 8 to 10 minutes response; (b) intermediate reaction of 20 to 40 minutes duration; (c) a prolonged freezing state for more than an hour. In mice with a prolonged response the possibility of inducing a further reduction in O_2 consumption by re-exposing them to the shadow before recovering was tested with negative results.

The magnitude of the metabolic depression under alarm was seen to be

Fig. 2 Varying duration of the metabolic depression in different deer mice alarmed by rotating shadow: short, intermediate and prolonged.



related to the ambient temperature (T_A), being reduced by only 10 to 15% above 30°C but by an average of 50% at 5 to 7°C (Fig. 3). This relates to the higher metabolic level at low T_A as normally observed in homeotherms. Undisturbed deer mice responded to a lowering of T_A by increasing heat production by 0.21 cc $O_2(g \cdot hr)^{-1}$ for every °C decrease in T_A. This value compares well with the range of 0.180 to 0.254 cc $O_2(g \cdot hr°C)^{-1}$ described for six other geographic races of the same species (Hayward, 1965; Gilman et al., 1950; Murie, 1961). The basal metabolic rate was 25 to 27% higher than reported values for the subspecies gambeli, nebrascensis and austerus (McNab and Morrison, 1963; Hayward, 1965) but during alarm the mean O_2 consumption at 30°C was 11% lower than the minimum values reported for three other strains of the same species (Cook and Hannon, 1954). Changing the speed of rotation of the overhead shadow had no apparent effect on the development of the alarm reaction, as shown in Fig. 4 for speeds of 10 up to 100 RPM.

The duration of the visual stimulus also had little or no influence on the metabolic response of the deer mice which in general showed the same response form when the disc was rotated for different lengths of time. In some animals a single pass of the shadow was enough to trigger a freezing response of several minutes. Figure 5 shows the metabolic behavior in one animal when stimulated for 5, 20 and 105 minutes. Simultaneous records of T_B show also that the degree of hypothermia during the alarm state does not depend on the duration of the stimulus but mainly on the reduction in heat production and on T_A. At the same

Fig. 3 Oxygen consumption vs. T_A in undisturbed (solid line) and alarmed (broken line) deer mice after 5 to 10 minutes stimulation by rotating shadow at 30 RPM. Numbers in circles indicate number of animals; vertical lines, range of values; open and closed diamonds, SD and SE.

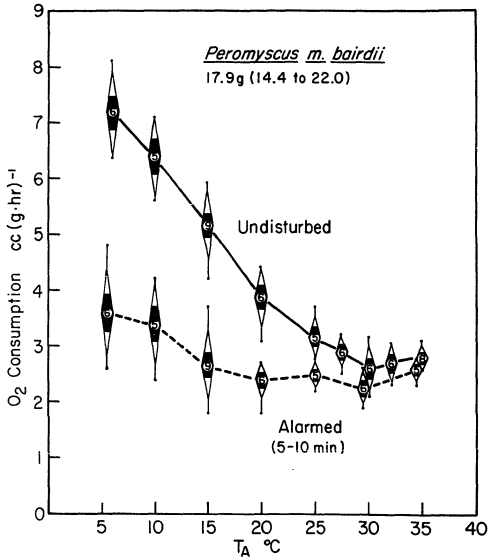
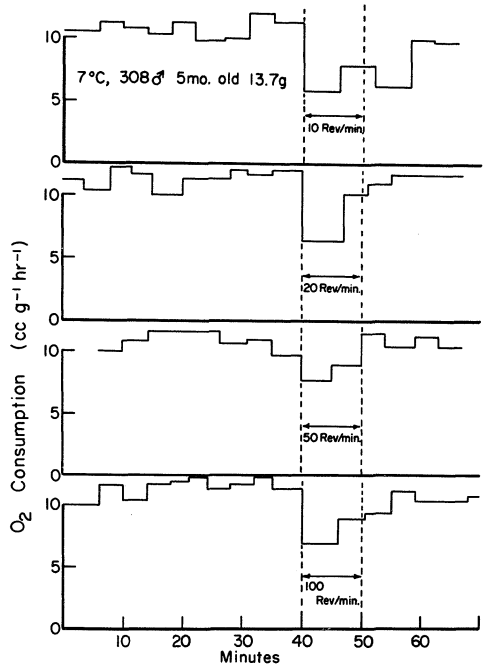


Fig. 4 The effect of different speeds of shadow movement on metabolic depression.



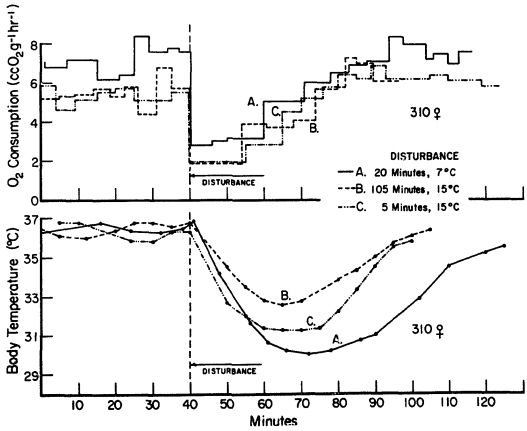
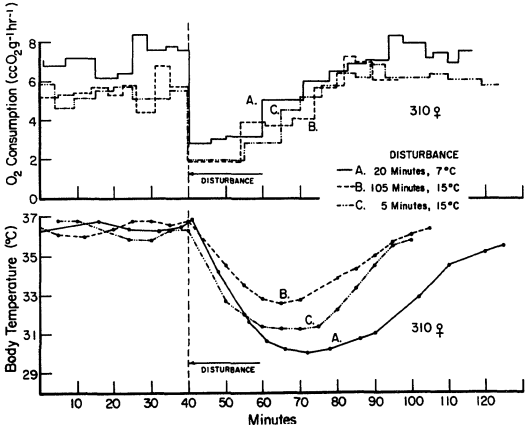


Fig. 5 The effect of stimulus duration on metabolic depression and the development of hypothermia.



T_A, rotation of the disc for 5 minutes caused a 4.9°C drop in T_B, and a comparable decrease of 4.2°C was observed when the moving shadow was projected for more than an hour. As shown in Figure 6, with the same stimulus at a lower T_A (5° vs. 15°C) hypothermia developed more rapidly (0.22° vs. 0.15°C/min) and the period of involvement was extended.

Of respiratory changes occurring during the alarm reaction, the most evident was a marked reduction in tidal volume to less than half of the resting values in undisturbed conditions. This shallow breathing was accompanied by an increase in respiratory frequency mostly at the end of the freezing period and immediately thereafter. The maximum increase in frequency was 85%, but in many cases the increase was considerably less.

O_2 consumption per breath follows the changes in tidal volume, decreasing from about 10 microliters before alarm to less than 4 microliters at the middle of the freezing period. Due to the relatively larger reduction in tidal volume as compared with the increase in frequency, the total ventilation diminishes during alarm as shown in Figure 7. The increase of ventilation during recovery from the freezing state did not result in an immediate increase in O_2 consumption but only after a delay of about 7 minutes.

As soon as the visual stimulus was presented, a strong bradycardia developed that closely correlates with the decrease in O_2 consumption (Fig. 8). Resting heart rates of 650 and 800 beats/min dropped to 200-280/min during the first minutes, rising thereafter to pre-exposure levels to parallel O_2 consumption.

Fig. 6 The effect of T_A on the development of hypothermia.

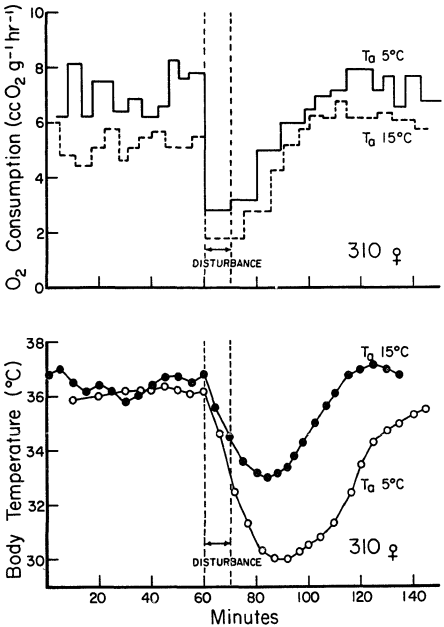


Fig. 7 Ventilatory responses in alarmed deer mice.

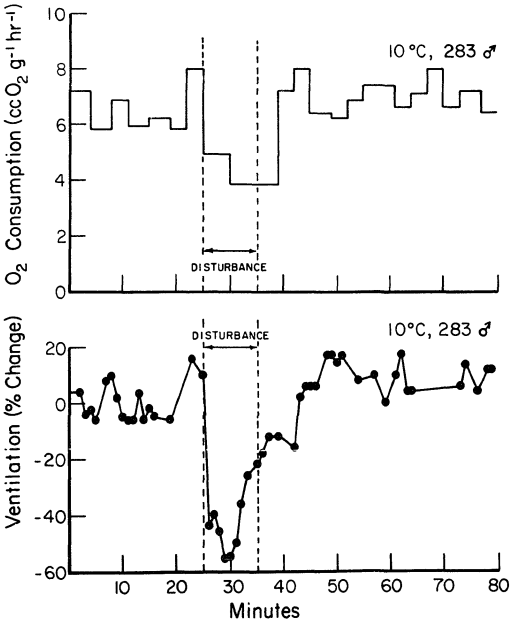
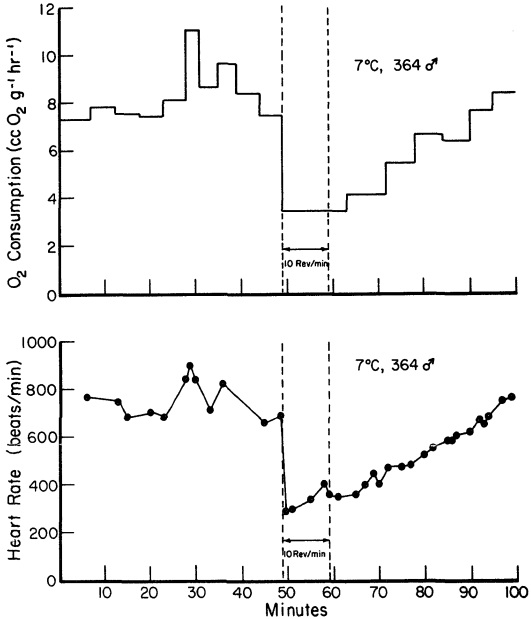


Fig. 8 Metabolic depression and bradycardic response to alarm in freezing of intermediate duration. T_A 7°C.



The relationship between heart rate and O_2 consumption at different conditions is illustrated in Figure 9. Values of the oxygen pulse varied between 2.5 µl/beat under deep alarm and 3.0 µl/beat during activity. During recuperation from the alarm state, the O_2 pulse was only 11 to 12% higher than initial control values at the same T_A, while a decrease of similar magnitude was observed when entering the alarm state.

Some individuals did alarm when the observer approached the metabolism chambers without interferring with the light path, and sound was considered as a possible stimulus to elicit the freezing response, but other indiviudals responded with an increase in O_2 consumption. To confirm this effect, a sonic stimulus of 20 KHz was remotely applied to the water tank after the animals had recovered from a previous visual stimulation. The response to each type of stimulus are illustrated in Figure 10. The increase in O_2 consumption with noise showed that Peromyscus maniculatus bairdii does react to other stimuli in the more common way of increasing heat production when disturbed.

DISCUSSION

The decrease in O_2 consumption in response to an object moving overhead was not correlated with the shape nor with the speed of the object. Similarly, chipmunks respond to shapes ranging from discs to T's with the same motor patterns and with the same intensity (Müller-Schwartze et al., 1971).

During the freezing response the magnitude of the metabolic depression

Fig. 9 O_2 consumption and heart rates measured at T_A 5°C under different conditions. Oxygen pulse indicated by arrows at normal (closed circles) and alarmed (open circles) states.

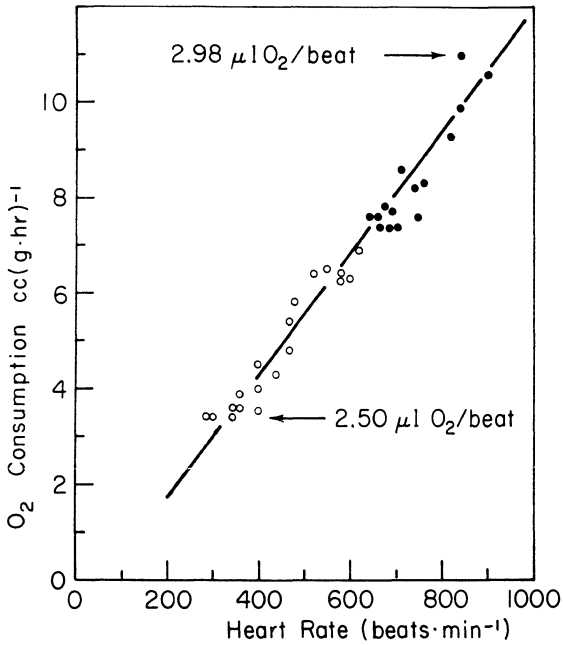
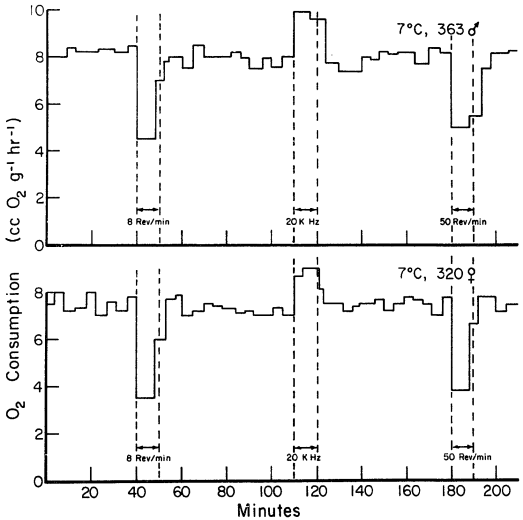


Fig. 10 Contrasting effects of visual (shadow movement) and acoustical (20 KHz) stimulation on the metabolic response of two animals. T_A 7°C.



was inversely related to T_A. At thermoneutral T_A, the 10 to 15% decrease may suggest a "real" basal value to the researcher not aware of the alarm reaction, but below 25°C the drop in heat production to 50% or less of the requirements for temperature regulation resulted in marked hypothermia. At 10°C our undisturbed deer mice were able to maintain a normal body temperature for many hours, but "lethargic" deer mice who do evade capture (alarmed?) have been reported at the same ambient temperature (Hart, 1953).

The duration of the alarm state varied between animals but was rather constant for each individual. A similar variation in susceptibilities to tonic immobilization has been observed in guinea pigs (Bayard, 1957), but in contrast to the training effect reported for this species on the duration of the hypnotic state (Liberson, 1948), no change was observed in our Peromyscus after repeated stimulation. Durations of torpor in P. m. sonoriensis of up to 7 to 10 hours have been reported to be independent of T_A (Morhardt, 1970a) and in a similar manner the duration of the freezing reaction was little affected by T_A which mainly influenced the depression in O_2 consumption and consequently the development of hypothermia.

Rewarming rates in different species of torpid pocket mice vary between 0.16 and 0.40°C·min^{-1} (Hayden and Lindberg, 1970). At 15 to 5°C alarmed deer mice cooled at rates of 0.17 to 0.36°C·min^{-1}, but spontaneous rewarming was in general somewhat slower at 0.07 to 0.21°C·min^{-1}. By contrast, the T_B in white-footed mice entering torpor, falls at only half the rate of the increase during rewarming. Different

postural configurations while entering torpor and while freezing not only can account for differences in cooling and warming but also suggest the contrast between the two phenomena of torpor and freezing. Torpid deer mice at 5 to 10°C showed T_B as low as 13 to 17°C (Morhardt, 1970a), but our animals in deep alarm at the same T_A seldom showed body temperatures below 28°C.

Respiratory frequency in torpid P. m. sonoriensis has been shown to decrease to 20 breaths/min or less (Morhardt, 1970b) but during the alarm response respiratory frequency was 10 times as high. A diminishing tidal volume resulted in a decrease of ventilation. Rapid, shallow respiration has also been reported in different rodent species after exposure to a strange environment (Guyton, 1947).

A gradual drop in heart rate from 700 to 200/min during 3 or more hours has been ovserved in torpid P. leucopus (Gaertner et al., 1973). In contrast, the bradycardia of freezing although of similar magnitude occurred immediately after the presentation of the optical stimulus indicating strong parasympathetic activity. Of probable similar nature, a deep baroreflexive bradycardia associated with a 60% reduction in O_2 consumption has been described in squirrel monkeys as a response to the pressor effect of phenylephrine (Wasserstrum and Herd, 1973). The depression of heart rate did not correlate with the T_B; in fact, the deepest bradycardia occurred within seconds after the initiation of the alarm reaction in normothermic animals and the return of the heart rate to normal was initiated while T_B was still falling or in maximum

hypothermic conditions.

While in man the oxygen pulse increases with the heart rate, it was not much affected in deer mice by different levels of heat production nor during the freezing state. In a series of rodent species, P. californicus has also been characterized by the relative independence of oxygen pulse and heart rate (Morhardt andMorhardt, 1971). Both bradycardia and the diminished ventilation occurring during the alarm reaction may explain a shortage of oxygen to the tissues and the consequent development of hypoxic rates of heat production.

Microtus agrestis and Clethrionomys britannicus have been reported to freeze or to flee and then freeze in response to the movement of objects overhead, according to the ongoing behavior at the time of the stimulation. Walking animals were more likely to flee than still animals who showed immediate freezing (Fentress, 1968a, b). Due to our different experimental conditions, walking was certainly restricted but some animals engaged in different moderate activities like grooming, eating or moving, were seen to freeze as well as when sitting still.

Whether or not a similar response to the shadow of a predator occurs in nature can only be hypothesized. The fact that Peromyscus has been observed to be active in dim light but not in darkness (Kavanau, 1967, 1968), does not rule out the possibility of such an occurrence during their crepuscular peaks of activity (Kavanau, 1962). Although the value of death-feighning as defense against predation has been questioned mainly on the basis of lack of evidence (Gilman et al., 1950), owl predation on moving or wandering Peromyscus has been observed to be five times

higher than in stationary individuals (Metzgar, 1967). If some degree of protection is to be gained by freezing, this mechanism might be expected to be of broader occurrence. Nevertheless we have been unable to elicit a similar response in another sub-species, P. m. borealis (from Alberta) which raises the question as to whether this difference in behavior is related to aerial vs. terrestrial predation in their respective habitats.

SUMMARY

Marked physiological reactions accompany the "freezing response" of deer mice, Peromyscus maniculatus bairdii, to overhead shadow movements. As much as 60% decrease in ventilation and in oxygen consumption and a bradycardia to less than 1/3 the normal heart rate resulted immediately upon the presentation of the stimulus. Progressive hypothermia then developed as a consequence and in proportion to the deficit in heat production, in turn influenced by the ambient temperature at which the alarm reaction was elicited. The duration (8 minutes to longer than one hour) as well as the intensity of the response as indicated by the depression of metabolism, was unaffected by the duration of stimulation (from 5 to 90 minutes) nor by the speed of the moving shadow (rotation of a disc from 9 to 100 RPM).

The rate at which body temperature fell, the rewarming rates, the respiratory frequency and the sudden bradycardic response all charcterized the freezing reaction as a different phenomena from torpor which has also been reported to occur in this and other species of rodents.

A different sub-species, P. m. borealis did not respond to the overhead movement by freezing, suggesting that this possible predator-defense mechanism may not be of general occurrence among rodents.

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CONCLUSION

The rate of oxygen consumption in homeotherms can be greatly modified in response to a variety of factors. Cold exposure, day-night cycles, solar radiation, wind velocity and oxygen shortage are some examples of environmental modifiers. And feeding and reproduction illustrate some of instinctive origin. Of physical nature, changes in body surface insulation or in overall thermal conductance modify the requirements for temperature regulation. Complicating elements are chronic infections such as Klossiella, causing a marked deterioration on the maximum metabolic capability but easily overlooked at low metabolic loads. Endogenous factors include endocrine and enzymatic changes, sex and aging. Behavioral and emotional reactions may sometimes counteract but other times, exacerbate the individual's response to one or more factors. Facing this multiplicity of metabolic modifiers the student of bioenergetics aiming to characterize specific responses has to isolate the effects of a given factor from the influence of the rest.

During the experimental phase of this work environmental modifiers such as temperature and P_{O_2} have been carefully controlled. Some unavoidable but low and monotonous background noise caused by water stirrers and cooling units was always present, but the general noise level during the tests was kept at a minimum.

The metabolic chambers and cages were washed in hot water or steam before each run to remove odors remaining from previous tests. Wherever possible, young adults were selected to confer some degree of homogeneity

to the group. Acclimation to controlled laboratory conditions for several weeks prior to the metabolic tests have undoubtedly helped in reducing the variation of the response.

Behavioral and emotional reactions have been more difficult to control. Here, the observer can do little to modify the restless behavior found in some species such as lemmings and voles, but only wait patiently for the most favorable periods of diminished activity. In some cases leaving the metabolic cages overnight inside the larger maintenance boxes at the animal quarters have led to its spontaneous acceptance.

In spite of these controls and precautions assurance of absolute isolation of the studied metabolic modifier is obviously impossible. Nevertheless, the elaboration of expressions describing the relations between normoxic and hypoxic metabolic rates have required to have on hand consistent data, suitable for quantitative analysis. Thus interference from undetermined factors may have been truly minimized.

The quantitative description of the metabolic effects of hypoxia under different metabolic loads may have initially appeared somewhat confusing to the reader due to the introduction of a few new symbols such as M, M_o, M_{max} and α which were believed necessary for an unambiguous identification of different metabolic stages; also by the introduction of a new unit of metabolism designated "met". Representing multiples of the standard metabolic rate for mammals, the met unit not only allows direct comparison of animals of different weight but it also has the advantage of being more meaningful (in the biological sense)

than current units. Thus, a value of 2 met indicates a low metabolic effort in a mouse as well as in a man, while by comparison, the expression 2 cc O_2/g·hr represents basal metabolism for the mouse but maximum metabolic effort for this writer.

Elicitation of maximum O_2 consumption in a He-O_2 atmosphere was accomplished in a much easier and less traumatic way than by conventional procedures. Thus, this technique may become the method of choice for such type of measurements. The metabolic increase of our rodents when exposed to the He-O_2 ranged from 40% in the hairless mice up to 130% in C. ducilla, but in the redpoll the increase was 160%. Whether or not the largest metabolic response was due to species-specific differences or to a more general characteristic of the insulative properties of feathers as compared with fur, remains to be proven.

An immediate reduction in ventilation, heart rate and O_2 consumption was elicited in our deer mice when visually disturbed by a moving shadow. This type of response was consistently observed in the subspecies P. m. bairdi (= bairdii) but not in P. m. borealis. It was then hypothesized that this difference in behavior could be related to aerial vs. terrestrial predation in their respective habitats. Subsequent experiments in other species showed that only Calomys ducilla and Ochotona rufescens responded to the visual disturbance in a similar way to P. maniculatus bairdi. Although native to three different continents these three species of mammals share a common trait, the selection of open habitats. In an environment as such where movement of the prey is the usual cue for aerial predators, the freezing behavior should be of

survival value. The fact that woodland species (Clethrionomys rutilus) and brushland species (Octodon degus) did not freeze upon the presentation of the stimulus, raises interesting questions on ecological-behavioral relationships.

A number of basic and yet unanswered questions have grown out of this study. Among these questions are the following: At a P_{O_2} below P_c, why does a rodent allow O_2 consumption to fall while it still has unutilized metabolic capability? What are the specific physiological or morphological characteristics that determine P_c and α; and which ones determine M_{max}? Does physical training modify the maximum response to cold? Can the He-O_2 technique give us a further insight on the character of seasonal changes in body surface insulation? Does the metabolic expansivity (scope for activity) change in response to season? How does M_{max} compare between subarctic and tropical species? Is the freezing behavior genetically determined or does it include a learning component? In the future search for these answers, the presently developed techniques will be of much value.